

acta ophthalmologica

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Poul Brændstrup

died January 26 1980 67 years old This is a very great loss for Scandinavian ophthalmology and especially for *Acta Ophthalmologica* For thirty years Professor Brændstrup was the mainstay of *Acta* (Managing Editor 1950–1970 Chief Editor 1970–1975 Associate Editor to 1980)

Poul Brændstrup was professor of the University of Copenhagen and head of the Municipal Eye Department of Copenhagen from 1957 He was president of the Danish Ophthalmological Society 1961–1963 and had a great number of honorary jobs because he had the full confidence of all his colleagues

He was Knight of the Dannebrog honoured with the Hjalmar Schütz medal and honorary member of the European Ophthalmological Society

His scientific works were profound and original His thesis Congenital Cataract and his works concerning ablato falciformis vitreous haemorrhage in newborn cystinosis optical aids for partially sighted central retinal vein thrombosis and senile cataract are still landmarks in ophthalmological research

Students and ophthalmologists will always remember him as a great and inspiring teacher

Poul Brændstrup died in his home a few hours after having returned from an evening party held with the staff of the hospital in celebration of his retirement

We will miss our dear friend and colleague

Mogens Norn

NEW YEAR - EDITORIAL

K K K Lundsgaard Acta and the Gold Medal

BY

MOGENS NORN

Professor K. K. K. Lundsgaard founded *Acta Ophthalmologica* and in 1931 on his deathbed he established the Acta Prize and Gold Medal. The motifs of the medal are described and the first eleven prize winners are mentioned.

Keywords: history K. K. K. Lundsgaard - periodicals Scandinavian Nordic

The cover and title page of *Acta* are decorated with the following words: K K K Lundsgaard *edit coepta*. This reminds us that Professor Lundsgaard founded *Acta Ophthalmologica*.

Who was this Dr. Lundsgaard? K. K. K. Lundsgaard (1867-1931) was the fourth Professor of Ophthalmology of the University of Copenhagen, Denmark (1909-1931). He had been christened Conrad Christian Carl but preferred K. K. K. C. C. C. to avoid being confounded with a relative (Lotttrup-Andersen 1952).

Dr. Lundsgaard was a very productive scientist. His 180 papers comprised practically all subjects of topical interest at that time, ranging from surgery, ocular lesions, and irradiation treatment to historical treatises and papers concerned with popular science. He introduced Crede's prophylaxis in Denmark and he is particularly remembered for ophthalmobacteriological publications.

Dr. Lundsgaard was well known for his organizing ability. He was one of the originators - and became President of - the Ophthalmological Society of Copenhagen. He was elected President of the Danish Medical Society and also of the International Association of Ophthalmologists.

Publication of the first Nordic periodical started in 1888 (Nordic Ophthalmological Periodical in Scandinavian Languages). It ceased on economic grounds with the fifth volume in 1892. Scandinavian ophthalmologic papers then had to be published scattered in foreign periodicals or in Scandinavian non-ophthalmological journals.

Dr. K. K. K. Lundgaard introduced such a Danish periodical (*Dansk Klinik*) which however only existed for two years (1909–1911).

In 1911 at the suggestion of Dr. Lundgaard among others a Scandinavian literature ring was established. The ring ceased to exist in 1975 after having sent more than 1500 reprints from Scandinavian authors to the members of the ring.

This was no satisfactory solution however. Dr. Lundgaard therefore at a society meeting in 1919 (Odense) began working for the introduction of a Nordic periodical published in languages of international currency. A subscription was started (among 40 Danish ophthalmologists about 50 per cent yielded from 100.00 to 1000.00 Danish crowns each) and he ventilated his idea to the Fifth Scandinavian Congress in 1921. Thanks to the enthusiasm and support with which he met among his Scandinavian colleagues the first issue of *Acta Ophthalmologica* was published in 1923. In this connection there is particular reason to mention Dr. Emil Enroth and Dr. V. Gronholm both Helsinki, Finland; Dr. Sigurd Hagen and Dr. Ingolf Schiøtz both Oslo, Norway; Dr. Fritz Ask Lund and J. W. Nordenson Uppsala, Sweden; Dr. Henning Rønne, Copenhagen, and as founder and first chief editor Dr. K. K. K. Lundgaard, Copenhagen, Denmark. The latter's private address (Lundsgade 6, København) is seen on the first title page.

On August 4, 1931 Professor Lundgaard – just over three weeks before he died – drew up a will in which he donated a fortune intended for a gold medal and a prize to be given at a Scandinavian congress to the author of the best article published in *Acta*. The will was addressed to Dr. Ejler Holm and Dr. H. U. Møller, his two successors. The will was rendered translated into German in *Acta* (H. U. Møller 1936).

We have kept the rough draft with its corrections and the hand written copy. Prof. Lundgaard signed the rough draft twice with a shaking hand. This was further evidence of Professor Lundgaard's interest in the continued publication of *Acta* and his desire to encourage future research workers.

The medal is of gold weighing 59.5 g. It is the same size as the Copenhagen University gold medal and carried out by Harald Salomon, the Royal Mint. According to the testator's wish the obverse of the medal is decorated with two owls (Fig. 1) – motif from a painting by Adrian Pietersz. v. de Venne (born in Delft, Holland, 1589, died in 1662). The small oil painting is still found at *Statens Museum for Kunst* (the State Art Gallery, Copenhagen) where Prof. Lundgaard was pleased



Fig 1

Obverse of the Lundsgaard gold medal showing two skating owls wearing spectacles

to see the owls wearing spectacles and enjoyed their remark "How well we are suited to each other" (*Hoe dienem wy by een*) (catalogue 1922 No 206 from Fredensborg 1905 wood 26.5 x 18 a skaung owl couple in peasant dress)

On the reverse of the medal (Fig 2) should be impressed the words *Acta Ophthalmologica Price* at the testator's wish Dr Ejler Holm (1904) found a spare space for a small relief representing Dr Lundsgaard's profile. Note however that it decorates the reverse side of the medal!



Fig 2

Reverse of the Lundsgaard gold medal with relief of Prof. Lundsgaard

Table I

Winners of Prof. A. A. A. Lundsgaard's gold medal and prize instituted at the Ninth Scandinavian Congress in Stockholm 1933

Gold medalist/subject	Congress	Place	Year	Acta ophthal
Ragnar Granit Helsingfors Finland Electrophysiology retina optic nerve	X	Copenhagen	1938	16 638 1938
Thore Lie Thomassen Oslo Norway Glaucoma	XIII	Goteborg	1934	32 732 1934
Arne Huggert Umea Sweden Pore size in trabecular meshwork in chamber angle	XV	Oslo	1960	39 748 1961
Bengt Rosengren Goteborg Sweden Retinal detachment surgery	XVII	Stockh	1963	44 496 1966
Torstein Bertelsen Bergen Norway Fibrilopathia epitheliocapsularis	XVIII	Copenhagen	1967	46 612 1968
Arvo Oksala Turku Finland Ultrasonography in Ophthalmology	XIX	Bergen	1969	48 832 1970
Torsten Arakau Lund Sweden Regulation of the intraocular pressure	XX	Rekjavik	1971	Suppl 120 92 1973
Mogens Vorn Copenhagen Denmark Vital staining of cornea conjunctiva	XXI	Helsinki	1973	Suppl 123 943 1974
Niels Ehlers Århus Denmark Graft thickness after penetrating keratoplasty	XXII	Lund	1973	Suppl 125 59 1973
Martin Daxanger Oslo Norway Suspensory apparatus of the lens	XXIII	Copenhagen	1977	56 833 1978
Ole I Aasen Copenhagen Denmark Continuous tonometry	XXIV	Oslo	1979	57 737 1979

At first the gold medal cost 337 34 Danish crowns and the medal stamps kept at the Royal Mint Copenhagen 1500 crowns. The prize amounted to 2000 00 crowns a large sum at that time.

The testator decided that the donation may be used to rescue *Acta* itself from possible financial difficulties. As soon as the financial circumstances permit the sum is to serve again for the payment of gold medals.

Professor Ragnar Granit Finland was the first to be awarded with the gold medal. On July 31 1938 Professor M. Vannas received the medal in Domus Medica Copenhagen on behalf of the prize winner who was prevented from coming.

During the Second World War and the first few years after the Nord. congresses were impossible to carry through. The next prize giving did not take place til 1951.

The economy of *Acta*, the Scandinavian spirit of unity and valuable publications have since permitted Prof. Lundsgaard's medal to be given away at nearly all Nordic congresses held so far (Table I).

Recommendations for the reward are to be undertaken by all Scandinavian professors in charge and the President of the Danish Ophthalmological Society. There are many publications outstanding enough as to deserve a gold medal. The choice may be difficult. Further unlike previously many articles and papers are nowadays published by two or more authors. But the medal can only be awarded to one author.

At the latest editor meeting in 1979 it was decided to raise the Lundsgaard prize appreciably.

Professor K. K. K. Lundsgaard was far seeing when he so keenly advocated publication of a Scandinavian ophthalmological periodical. At the first turn of the year *Acta* will complete its 57th birthday - a rather considerable age. *Acta* thrives well, its economy is in order. Nordic ophthalmological science is of an outstanding quality. Manuscripts are crowding and the number pages increases. No less than 14 supplements have been issued within the last three years.

It will be exciting to study the publications that will appear in *Acta Ophthalmologica* in the course of 1980.

Happy New Year!

Acknowledgements

My thanks are due to Mr. William Celsus Jensen, Art Historian, and to Professor Poul Brandstrup.

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ELECTRONIC SUBTRACTION METHOD FOR OPHTHALMIC PHOTOGRAPHY

BY

E JAANIO H ALANKO P J AIRAKSINEN H NIEMINEN and S LÄHDE

A commercial electronic subtraction unit originally intended for the study of roentgenograms was used to produce subtraction pictures of fundus fluorescein angiographies. No modifications were needed to make good quality subtraction pictures. The method is fast and inexpensive in contrast to previously described photographic methods. Certain limitations of the present equipment are discussed as well as some possibilities for further development of the apparatus. The method is especially suitable for studies on dynamic processes in the eye and it should prove valuable in the long term follow up of chronic diseases such as glaucoma and diabetic retinopathy.

Key words: diagnosis — fluorescein angiography — fundus photography — closed — circuit television

Ziedses des Plantes (1935) presented the principles of the subtraction method now in use in roentgenologic angiography. The main idea is to subtract the blackness values of one roentgenogram point by point from those of another using the same blackness scale to form a new print in which parts common to both originals are pictured only faintly and differing parts become enhanced. It is also possible to compare two frames from an angiography taken at different time lapses and to demonstrate the changes in vascularity that have taken place between the two pictures: the so called phase subtraction method (Demmger 1970).

Honuchi (1971) and Makabe (1972) applied photographic subtraction method for fundus fluorescein angiographies. Mikuni & Fujii (1972) used the autosubtraction method to produce a pseudo-relief effect, which helps to interpret the fine

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structure in angiographies. The subtraction method was found valuable in the assessment of fundus photocoagulation effects of the retinal edema and retinal vascular fluorescein leakage in central serous retinopathy.

The progress in modern videotechnology has made electronic subtraction methods possible. Electronic subtraction units based on closed-circuit television (CCTV) are used mainly in roentgenology. A common construction principle for electronic subtraction apparatus is the use of two vidicon type television cameras in sync to form video signals from the original pictures. The video signals are then amplified by logarithmic amplifiers (Ziedses des Plantes 1968) to preserve the original information of exponential intensity differences. Subtraction is achieved electronically by inverting the polarity of one of the video signals and then combining the two signals synchronously. The combined video signal now carries information from both original pictures with electronic subtraction and the result can be observed on a television monitor. The subtraction picture on the monitor can be changed continuously by the balance and contrast controls which enables quick and smooth study of different grades of subtraction between the original pictures.

Photographic subtraction methods are known to be slow, complicated and expensive (Deininger 1970, Ziedses des Plantes 1968) which greatly hampers their use in everyday clinical work. The subtraction method nevertheless showed great promise in principle and we decided to examine the use of electronic subtraction in ophthalmology since these methods were known to produce good results in roentgenology fast, effortlessly and at low costs (Deininger 1970, Ziedses des Plantes 1968).

Material and Methods

The case we chose to present was a 19-year-old female who had had prodromal transient scotomas in the right eye for half a year. At the time of the examination she had a permanent field defect of six days duration. Ophthalmoscopy revealed an obstruction in a branch of the superior temporal retinal artery and a corresponding retinal edema. Routine serial fundus fluorescein angiography was immediately performed. Later the obstruction disappeared leaving no other traces other than the permanent field defect. Figs 2 and 3 were taken at 17.1 second and 30.6 seconds after the injection of fluorescein as seen from the automatic time recorder on the right side in the pictures. They were used for subtraction directly in 18x94 cm copies which are routinely supplied with the angiography.

The copies to be subtracted were placed under each camera of Siemens M 707 A subtraction unit (Fig. 1) based on CCTV and necessary adjustments of the camera diaphragms were made. The pictures were then moved by hand until they almost

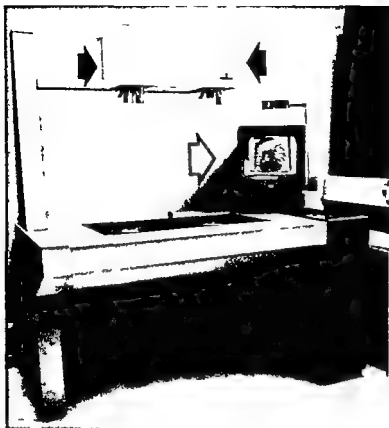


Fig 1

Siemens electronic subtraction unit M 707 A. The two video cameras and the television monitor are indicated by arrows. The camera at the left produces a normal video signal. The camera at the right produces an inverted (negative) picture.

coincided on the monitor and the fine adjustments of the masking were made by the use of the electronic overlapping controls. The correct balance setting was controlled by the use of test charts. Subtraction pictures were observed directly on the monitor. Pictures to be documented were photographed by a Hasselblad 500 C camera with a Zeiss Planar objective of 100 mm focal length using Kodak Tri X film and 1/5 second exposure time.

Results

The subtraction pictures were quite acceptable in sharpness (Fig. 4). Phase subtraction made it possible to observe minor changes of fluorescence pattern such



Fig. 2

Fluorescein angiograph of the fundus showing retinal arterial branch obstruction 171 seconds after injection. The picture is inserted electronically to its negative.

as the retrograde filling of the artery (Fig. 4). A pseudorelief effect like enhancement of contours was also easily obtained by slightly displacing the overlapping pictures—a phenomenon especially useful in the evaluation of the boundaries of retinal edema (Fig. 4). The changes of balance and contrast between the pictures also add new details of information.

A small part of the vein below the obstruction at the border of the retinal edema (Fig. 4, solid arrow) is toned lighter, proving that there has been a local change in the fluorescein concentration in this part of the vein starting from a small branch in the edematous area. The change in tone is so small that it is almost impossible to observe by comparison of the original angiography frames (Fig. 2 and 3).

The resolution in the subtraction picture is naturally inferior to that in the original pictures, but it was found quite sufficient for the present purpose. The use of higher magnification partly compensates for the lack of highest resolution and enables the study of interesting areas in minute detail.



F = 3

A later picture taken at 30.8 seconds. A normal television picture was obtained from this photograph

Discussion

The value of subtraction methods in ophthalmology has been evident after the works of Horuchi (1971), Mikuni & Fujii (1972) and Makabe (1972) but it has gained no wider clinical use. Their works were all done by photographic procedures with inherent limitations and it is our belief that this has been the major reason for the poor response that the subtraction method has won in clinical ophthalmology.

To our knowledge we are the first to use the electronic subtraction method for the interpretation and analyses of fundus fluorescein angiographies.

This method seems to be very suitable for the diagnosis of fundus angiographies. Minor changes for this commercial subtraction unit make it still more efficient for ophthalmic use. The changes we have introduced now include the use of zoom lenses which will provide variable magnification capability to both cameras to meet the possible discrepancy in the magnification ratio of the copies to be compared. With larger magnification it is also possible to compensate for the loss of distinctness in the TV picture.



Fig. 4

Electronic subtraction picture of Figs 2 and 3 as seen on the television monitor. C overlapping of the pictures in the central area. The boundaries of the retinal edema clearly shown as well as the retrograde filling of the obstructed arterial tree (open arrow) the filling of a small branch of the vein from edema area (asterisk). Solid arrow points local change in a vein otherwise evenly filled with fluorescein. The subtraction picture shows the changes that have taken place in the fluorescein distribution in the fundus during 13.7 seconds between the exposures of the two angiography frames.

The principles of subtraction require great uniformity of the pictures to be subtracted. Changing the photographic angle in fundus photography produces distortions which theoretically should be hard to overcome. In practice we found, however, that excellent subtraction pictures could be achieved with our method.

The electronic subtraction method will help in the diagnosis of routine fluorescein angiographies but we think that the most rewarding use will be found in the long term follow up of glaucoma, diabetic retinopathy and tumors of the eye. Comparison of pictures taken on different occasions will be faster and more reliable using subtraction. Minimal changes of various pathologic conditions in eye can be detected earlier and more easily. For recording purposes the subtraction picture can either be photographed from the monitor or the information can be stored

videotaping or by the use of special memory units. Colorcoding (Fisher 1958) which has been experimentally used in roentgenology may further facilitate perceiving small differences of contrast in the subtraction picture.

In a following paper we will report on glaucomatous optic disc changes demonstrated by electronic subtraction.

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DEMONSTRATION OF GLAUCOMATOUS OPTIC DISC CHANGES BY ELECTRONIC SUBTRACTION

BY

H. ALANKO, E. JAANIO, P. J. AIRAKSINEN
and H. NIEMINEN

We describe a new method, the electronic subtraction, for objective two-dimensional detection, demonstration and recording of glaucomatous optic disc changes. Siemens subtraction unit M 707 A, based on a double videochain and originally developed for the study of roentgenograms, was used. The results show that this technique is useful in demonstrating the progressive damage of neural tissue of the disc and associated alterations in the course of the vessels. Further development of the electronic subtraction method for glaucomatous optic disc evaluation is discussed.

Key words: optic disc - glaucoma - electronic subtraction - fundus photography

Glaucomatous visual field defects are preceded by subtle changes of the optic disc and these may constitute the first clinical sign that the intraocular pressure (IOP level) is not tolerated by the eye (Armaly 1969; Chandler & Grant 1963; Shaff 1969). Therefore a definite need exists for an objective and sensitive method *detecting and recording possible glaucomatous changes of the optic discs of patients with ocular hypertension and glaucoma.*

In this paper we report an electronic subtraction technique for the detection and demonstration of these changes. According to the principles of subtraction all parts which are common to original pictures with no difference in size, shape or black values fade towards the neutral grey, but the differing parts become enhanced in contrast to photographic methods. Electronic subtraction is fast and easy to perform. Details of electronic subtraction in ophthalmic photography have been presented in a previous paper (Jaanio et al. 1979).

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Material and Methods

For the present study optic disc stereophotographs of two female patients were selected on the basis of stereoscope findings. One patient, aged 77 years, had been treated for chronic open angle glaucoma for 15 years (case 1). The other patient, aged 77 years, with normal IOP and visual fields but suspect glaucomatous cupping of the left optic disc, had been followed for seven years without treatment (case 2). All stereophotographs were taken by the same photographer (H. N.) with a Zeiss Ikon camera and Allen stereo separator.

In case 1, three pairs of stereophotographs were available with a time interval of two years between the first and the second photographs and three years between the second and the third photographs. In case 2, we had two stereopairs taken 15 months apart. For subtraction, we used the right hand pictures of each stereopair. To avoid unequal magnification and contrast, new prints in the same enlargement scale and with equal blackness values were made from the original negatives. In case 2, the "earlier" picture was inverted electronically to its negative and subtracted from the "later" by a Siemens electronic subtraction unit M 707 A. Similarly, in case 1, the first picture was subtracted from the second and the third picture, and the second picture from the third one. Resulting subtraction pictures were photographed from the TV monitor of the Siemens M 707 A unit with a Hasselblad 500 C camera.

Results

Electronic subtraction shows the differences in two photographs taken of the same object at different times.

Fig. 1A to F

Fig. 1. A, B and C are the right hand pictures of the original stereopairs (case 1). Dates of photography: A in March 1970, B in May 1974 and C in April 1977. D, E and F are subtraction results photographed from the monitor of the Siemens M 707 A electronic subtraction unit. D is the resulting subtraction picture between A and B, E between B and C, and F between A and C.

D: Disappearance of a small haemorrhage, seen in picture A (open arrow), produces a clearly demarcated white area (solid arrow).

E: This picture shows the displacement of the small inferotemporal vessel. Solid arrow points at the original course (white stripe) and open arrow at the new course (black stripe) of the vessel.

F: The superior arterial branch in the optic disc shows a nasal (solid arrow) and inferior (open arrow) displacement. Pictures B, E and F show clearly the progressive enlargement of the cupping (i.e. loss of neural tissue) in the direction of the haemorrhage seen in picture A, in contrast to the unchanged superotemporal neural rim.



In case 1 the increasing white infero-temporal area demonstrates the progressive notching of the neural rim (Fig 1D, E and F). The gradual displacement of the disc vessels can be seen as deviating black and white stripes (Fig 1E and F). The light streak on the disc border at four o'clock position may indicate a change of size or position of the peripapillary pigmentation (Fig 1F asterisk).

In case 2 the lack of contrast indicates that no change had occurred in the contour of the neural rim or location of the vessels during the observation period (Fig 2C).

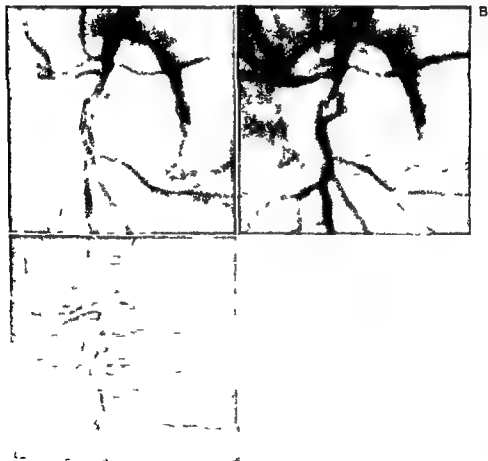


Fig 2A to C

Fig 2 A and B are the right hand pictures of the original stereopairs (case 2) photographed in February 1975 (A) and in May 1976 (B). C is the subtraction result between A and B. The unanimous neutral grey and absence of outstanding white areas indicate that no change in the optic disc had taken place during the observation period.

Discussion

The methods of detecting changes of the optic disc are inadequate. The most common procedure is the comparison of stereophotographs with ophthalmoscopic findings but more accurate, objective and faster means for clinical purposes are still lacking.

Stereophotogrammetry has been considered a possible solution and adaptations of analogue (Crock 1970, Jonsas 1972, Saheb et al 1972, Portney 1974), analytic (Portney 1975, Kraljic 1974) or digital (Hostler et al 1974) techniques for ophthalmology have been reported. Crock (1970) supposed that photogrammetry will pass into routine practice but a useful clinical application is yet to come. The eye as a part of the imaging system makes stereophotogrammetry of the optic disc complicated (Kraljic 1974, Dowman & Elkington 1974). Moreover, the atypical imaging geometry of the commercially available fundus cameras does not fulfil the photogrammetric requirements (Kraljic 1974).

In contrast to stereophotogrammetric methods, electronic subtraction is basically two dimensional and gives no direct information of the cup profile and volume but selectively produces information of the changes of the neural rim width which is of major importance in the management of glaucoma.

For electronic subtraction no stereophotographs are needed. Simple black and white pictures of the disc are adequate and their quality is generally superior when compared with stereoscopic ones. In addition, photographing through a smaller pupil is possible. If the subtraction unit is not fitted with zoom-optics, the enlargement scales of the original photographs must be equal.

The information of the subtraction picture in the monitor is in digitized mode and this enables further handling of the data and the quantitation of the neural rim changes by digital micro computer solutions.

The electronic subtraction method is objective and fast and gives a permanent record in the study of photographs of the same optic nerve head taken on different occasions. The sensitivity of the method and the quantitation of the results need further studies. We think that the electronic subtraction method may prove a valuable contribution to the evaluation of glaucomatous optic disc changes.

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FINDINGS ASSOCIATED WITH GLAUCOMATOUS VISUAL FIELD DEFECTS

BY

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1511 persons 50 to 70 years-old and making up 77% of a population in which every case of chronic primary glaucoma with visual field defects had remained untreated were comprehensively examined using standardized methods (including automatic perimetry) and strict diagnostic criteria in order to assess different methods of identifying the glaucomatous members. The prevalence of manifest glaucoma (1%) was unaffected by systemic haemodynamic and vascular disorders. In eyes with manifest glaucoma simplex the dispersion of pressures was small and the mean only about 5 mmHg higher than in all eyes. Seven out of ten subjects with disc haemorrhages also had glaucomatous visual field defects. Other findings causing a suspicion of glaucoma could – as defined here – not have been used to ascertain a diagnosis of manifest glaucoma. In 13 out of 15 subjects with glaucomatous visual field defects an enlarged cup was noted at the initial examination. No other method (except automatic perimetry) could – as applied here – have been used to exclude the possibility of a manifest glaucoma with an acceptable degree of certainty.

Key words: glaucomatous visual field defects – disc haemorrhages – enlarged optic cup – increased intraocular pressure – anterior segment pathology – family history

On the basis of methods used so far only a small minority of all persons going to develop glaucomatous visual field defects can be identified with a degree of precision sufficient to warrant therapeutic intervention prior to the onset of irreversible function loss (Armaly 1969–1977). For the time being we must dire

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our efforts towards a more effective detection and control of manifest glaucoma (= secondary prevention) For this purpose

"(I) Development of techniques for diagnosing field defects much earlier when randomized controlled (therapeutic) trials might be considered ethical and

"(II) More detailed study of the factors related to glaucomatous field defects in the general population"

have been proposed as probably the most useful lines of research (Cochrane et al 1968)

The prevalence of manifest glaucoma seems to be much lower than commonly thought (Hollows & Graham 1966) It rises steeply with age but new cases seem to occur at about the same rate from the early fifties on (Bengtsson in press) The incidence is therefore always low – about 0.1% which means that on an average one new case can be expected every year per 1000 persons aged 55 years or more In order to obtain a representative material of manifest glaucoma in early stages a sensitive visual field screening has therefore to be repeated at short intervals in large numbers of subjects comprising all – or a truly representative sample – of the population In order to facilitate an assessment of factors believed to be associated with the occurrence of glaucomatous visual field defects in the general population a more comprehensive examination of each subject should be added to the actual detection of such defects

A study along these lines but on a limited scale was started at the Dalby Community Care Centre (in Southern Sweden) as soon as the most important prerequisite – the automatic perimeter (Hetjl & Krakau 1973a b Krakau 1978) – had become available a few years ago When it was found that 80% of all cases of glaucoma (simple or not) with visual field defects and in fact every case of manifest glaucoma simplex in the population had remained untreated right up to the initial survey it was realized that an in all essentials representative material of manifest glaucoma in comparatively early stages had been obtained already at the first attempt The present report of the findings at the initial survey was therefore considered to be justified

Material

All persons born 1907–1921 and resident in the district serviced by the Dalby Community Care Centre were listed in December 1976 The list was arranged according to the residential addresses and kept up to date by means of weekly reports from the authorities on removals and deaths Following this directory the

inhabitants were contacted in rotation during 1977 and 1978 and offered repeat ophthalmological examinations. Attempts at persuasion were avoided and persons able and willing to attend within a few weeks were included in the survey.

One person known to be blind and 24 patients subjected to antiglaucoma therapy were not invited. There were only five cases with field defects (in congenital and four secondary glaucomas) in this group.

Out of 1938 invited persons 1511 (78%) took part in the survey. The attendance was largely independent of age and sex.

Methods

A brief questionnaire concerning medication and general eye symptoms was mailed together with the appointment notification. At the Centre the answers were discussed and supplemented with a medical history comprising specific questions concerning diabetes, vascular diseases (thromboses, haemorrhages and ischaemia), haemodynamic crisis, prodromal symptoms, ocular trauma, uveitis and family history.

Sphygmomanometric measurements of the systolic blood pressure, determination of the visual acuity, subjective refraction, automatic perimetry, fundus photography, indirect ophthalmoscopy, slit lamp examination and Goldman tonometry were attempted in every case. Apart from the perimeter, conventional equipment was used in a fairly standardized manner. Perimetry and photography were conducted by two alternating assistants – ophthalmoscopy, slit lamp examination and tonometry by the author. Perimetric data handling was entirely automatic; other data were immediately recorded on special forms. Transfer to magnetic tape and further processing were performed at the Computer Centre in Lund.

The systolic blood pressure was measured on the right arm with the subject sitting position.

At ophthalmoscopy disc haemorrhages, large cups, notching of the rim (Sp 1978) and vertically oval cups were particularly noted. No attempt was made to estimate the size of the disc.

The slit lamp examination was directed towards the detection of niaschen disorders, pseudoexfoliations, pigment dispersion, narrow angles, rubeosis iridis, posttraumatic and (post)uveitic signs.

Detailed descriptions of automatic perimetry and optic disc photography as applied in the present study have been given elsewhere (Bengtsson & Krakau 1979).

Criteria

A *glaucomatous visual field defect* (GVFD) was inferred when an "automatic" visual field defect consistent with glaucoma but not explained on other grounds had been reproduced at follow up

A person was considered to suffer from *manifest glaucoma* when a GVFD and a corresponding loss of neural tissue in the optic nerve head co-existed in the same eye. This judgement was based on a subsequent comparison of "automatic" field charts and fundus photographs taken at the survey (but not necessarily in agreement with the first impression obtained at the initial examination)

A *family history* of glaucoma was accepted when at least one close relative was reported to have been subjected to antiglaucomatous treatment

A report of *diabetes vascular disease* or a *haemodynamic crisis* was considered notable only if it was based on a clinical judgement prior to the survey

An *enlarged cup* was suspected if a large cup, a notching of the rim or a vertically oval cup was observed with the ophthalmoscope at the initial examination

A *disc haemorrhage* on the other hand, was not recorded unless its transient nature had been confirmed by repeated photographs

The term *anterior segment pathology* was used to summarize disorders possibly obstructing aqueous outflow detected at the slit lamp examination. Gonioscopic findings were not included

Results

The visual field screening resulted in the detection of 20 visual field defects consistent with glaucoma and not explainable on other grounds at the time of the survey. One of these defects disappeared within a year and another turned out to be part of a homonymous one. The remaining 18 defects fulfilled our criteria for a *glaucomatous field defect*. Thus 15 cases (twelve unilateral and three bilateral) with previously unknown glaucomatous visual field defects were identified independently of other factors associated with glaucoma

The glaucomatous visual field defects were found in eyes with notching of the rim (8 cases), a large cup extending to the disc margin (6 cases) or a large cup with a localized undermining of the rim (one case). Thus all eyes with glaucomatous visual field defects also fulfilled our criteria for a *manifest glaucoma*.

The numbers of positive answers to our questions concerning *diabetes vascular diseases*, *haemodynamic crisis* and *antihypertensive medication* provided by all subjects and by subjects with glaucomatous visual field defects are listed in Table 1. A total of 674 such disorders and treatments were reported by 436 out of the 1511 subjects taking

Table I
Number of subjects reporting indicated diseases and medications at survey

	Among all subjects N = 1511	Among subjects with manifest glaucoma N = 15
Diabetes	64	0
Vascular diseases	171	2
Haemodynamic crisis	44	1
At least one of the above diseases	244	3
β -blockers	138	1
Diuretics	213	1
Other antihypertensives	44	0
At least one of the above medications	295	3
At least one of the above diseases and/or medications	436	4

part in the survey. Similarly six such diseases and medications were reported four out of the 15 persons in whom glaucoma with field defect was detected at survey. Even in the different subgroups the prevalence of manifest glaucoma is very much the same as in the whole material (1%).

The distribution of *systolic blood pressures* in subjects with manifest glaucoma closely followed that in all subjects. The means were 150 and 153 and the standard deviations 22 and 23 mmHg respectively, but the difference was not statistically significant (Student's $t = 0.5$).

Similarly the distribution of *glass refractions* in eyes with glaucomatous visual field defects closely followed that in all eyes. The means were +0.7 and +0.9 and the standard deviations 1.3 and 1.9 diopters respectively, but the difference was not statistically significant (Student's $t = 0.4$).

The *visual acuity* was reduced (< 0.9) in at least one eye in 120 persons (8%). About 15% reported *eye trouble* in the questionnaire. Among 15 persons with manifest glaucoma two had a reduced visual acuity in one eye caused by nuclear cataract and by a posterior vitreous membrane on the anterior surface of the lens and two reported eye symptoms caused by the cataract mentioned above and by a vertical squint.

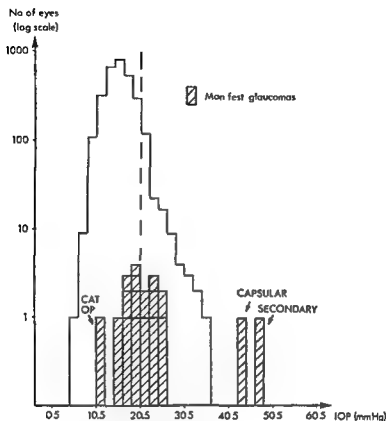


Fig 1

Frequency distribution of intraocular pressures in all eyes and in eyes with glaucomatous visual field defects detected at the survey

The distributions of intraocular pressures in all eyes and in eyes with manifest glaucomas detected at the survey are given in Fig 1. If one aphakic eye and two eyes with anterior segment pathology are disregarded, the dispersion of pressures of eyes with glaucomatous visual field defects was small and the mean only about 5 mmHg displaced to the right ($\bar{X} \pm \text{SD} = 20.8 \pm 2.9$ mmHg).

In ten eyes with disc haemorrhages, seven of them with manifest glaucoma, the intraocular pressures were similarly assembled but even less displaced to the right ($\bar{X} \pm \text{SD} = 18.5 \pm 2.9$ mmHg). The mean pressure was still significantly higher than in eyes of persons without manifest glaucoma ($\bar{X} \pm \text{SD} = 15.5 \pm 3.1$ mmHg).

In "normal" persons (without glaucomatous visual field defects) the intraocular pressures were significantly more dispersed and their mean significantly higher in eyes with enlarged cups ($\bar{X} \pm \text{SD} = 16.8 \pm 4.5$ mmHg) than in others eyes ($F = 2.0$, Student's $t = 3.0$). In eyes with

Table II
Total number of subjects with indicated findings at survey

	Among all subjects N = 1511	Among subjects with G\&FD N = 15
Disc haemorrhage	10	7***
Enlarged optic cup	114	13***
IOP > 20.5 mmHg	110	7***
Anterior segment pathology	68	2 ⁿ
Family history	73	4 *
At least one of the above findings	313	15 **

ns = not significant

** = significant ($P < 0.01$)

*** = highly significant ($P < 0.001$)

(post)uveitic signs the intraocular pressures were widely dispersed ($so = 8.5$ $n = 11$) and a small group contained both the lowest and two out of the six highest pressures observed in eyes without glaucomatous visual field defects at the survey. Pseudoexfoliations were usually unilateral (20 out of 24 cases without glaucomatous visual field defects) and the intraocular pressure in the affected eye on an average 2 mmHg higher than in the fellow eye but not significantly higher than in eyes of normal persons without pseudoexfoliations (since the average of the fellow eye was somewhat lowered). Eyes with other types of anterior segment pathology as well as persons providing a positive family history had intraocular pressures with more ordinary distributions.

Persons with disc haemorrhages (B), enlarged optic cup (E), IOP > 20.5 mmHg (F), anterior segment pathology (G) or a family history (H) are considered as glaucoma suspects. The total number of subjects and the number of subjects with G\&FD presenting such findings at survey are given in Table II. In all five groups the frequency of previously unknown glaucomatous visual field defects was higher than in the whole material. The association with manifest glaucoma was statistically significant except in the group with anterior segment pathology but not very strong except in the groups with ophthalmoscopic findings. The number of persons with at least one of these five findings, i.e. the total number of suspects, was remarkably great (313 out of 1511 subjects = 21%) and accordingly the rate of cases with manifest glaucoma among all glaucoma suspects remarkably low (5%).

All types of findings which cause a suspicion of glaucoma were more common in women than in men. This sex difference, however, was insignificant and restricted to subjects with manifest glaucoma in case of ophthalmoscopic findings and large

dependent on the distribution of pseudoexfoliations (20 women and five men) in case of anterior segment pathology

The relative frequency of enlarged cups and high intraocular pressures increased with age. The occurrence of anterior segment pathology was independent of age. A family history of antiglaucomatous treatment was furnished comparatively seldom by older persons. The age differences were statistically significant for enlarged cups as well as for family histories and in accordance with current views on the matter for high pressures.

To generalize findings associated with glaucomatous visual field defects were more common in subgroups of the population characterized by a relatively high prevalence of manifest glaucoma. For any finding the variation in degree of association with glaucoma within the material was therefore less marked – and the statement of a single figure to describe the relation more justified – than if the reverse had been the case.

Table III

Number of subjects with indicated combinations of findings (findings not denoted may – but need not – be present) expected (assuming independence) and observed in the present study

	1		2		3	
	Total number of subjects		No of subjects without GVFD		No of subjects with GVFD	
	Exp §	Obs	Exp §	Obs	Exp §	Obs
BE	0.8	7	0.90	1	6.1	■
BF	0.7	2	0.21	0	3.5	■
BH	0.5	3	0.14	1	1.9	2
EF	8.3	24	6.95	18 *	6.1	6
EH	5.5	9	4.66	5	3.5	4
FH	5.3	6	4.75	4	1.9	2
BEF	0.05	2	0.014	0	2.8	2
BEH	0.04	2	0.009	0	1.6	2
BFH	0.04	1	0.010	0	0.9	1
EFH	0.40	■	0.391	0	1.6	2
BEFH	0.003	1	0.001	0	0.8	1

§ $\text{Exp } XY = n \cdot \frac{\text{Obs } X}{n} \cdot \frac{\text{Obs } Y}{n}$ $n = 1511$ (in col 1) 1496 (in col 2) or 15 (in col 3)

** Highly significant ($P < 0.001$)

B = Disc haemorrhage E = Enlarged cup F = IOP > 20.5 mmHg ■ = Anterior segment pathology H = Family history GVFD = Glaucomatous visual field defect.

Findings significantly associated with glaucomatous visual field defects i.e. disc haemorrhages enlarged cups IOP > 20.5 mmHg and positive family histories were markedly associated with each other and the observed frequencies of all possible combinations of them were higher than expected according to the opposite hypothesis (Table III 1). With one exception the association between enlarged cups and IOP > 20.5 mmHg in persons without glaucomatous visual field defects all such mutual associations were entirely explained by the associations of the pertinent findings with manifest glaucoma i.e. the observed frequencies of different combinations in persons with and without manifest glaucoma agreed extremely well the expected ones (Table III 2-3).

Table IV

Number of subjects with indicated combinations of findings (a dash denotes the absence of the finding in question). The number of subjects with glaucomatous visual field defects contained in each group is given within brackets.

B - - -	2 (1)
- E - -	79 (2)
- - F -	82 (1)
- - - H	59
Subtotal	222 (4)
B E - -	4 (3)
B - F -	
B - - H	1
- E F -	21 (5)
- E - H	6 (1)
- - F H	4
Subtotal	36 (7)
B E F -	1 (1)
B E - H	1 (1)
B - F H	
- E F H	1 (1)
Subtotal	3 (5)
B F F H	1 (1)
Total	262 (15)

B = Disc haemorrhage E = Enlarged cup
F = IOP > 20.5 mmHg H = Family history

Table V

Conditional probabilities of having a glaucomatous visual field defect if positive - $P(GVFD/X)$ - and of being positive if diseased - $P(X/GVFD)$ - with their 95% confidence intervals

	$P(GVFD/X)$	$P(X/GVFD)$
Disc haemorrhage	0.70 (0.35-0.93)	0.47 (0.21-0.73)
Enlarged cup	0.11 (0.06-0.19)	0.87 (0.60-0.98)
IOP > 20.5 mmHg	0.06 (0.03-0.13)	0.47 (0.21-0.73)
Anterior segment pathology	0.03 (0.00-0.10)	0.13 (0.02-0.40)
Family history	0.05 (0.02-0.13)	0.27 (0.08-0.55)

A major part of the glaucomatous visual field defects were accordingly found in subjects with a coincidence of two or more of the four findings significantly associated with a manifest glaucoma in the present population (Table IV). In subjects with only one good reason to suspect glaucoma on the other hand glaucomatous visual field defects were not much more common (1.8%) than in the whole material (1%) (Table IV).

To increase their comparability the frequencies in Table II were transformed into conditional probabilities (Table V).

- 1) of having the disease if positive $P(GVFD/X)$ = "the diagnostic specificity" and
- 2) of being positive if diseased $P(X/GVFD)$ = "the nosological sensitivity".

The basic numbers were small and the 95% confidence intervals wide (Table V) but the following observations were considered reliable enough to deserve mentioning.

Seven out of ten subjects with disc haemorrhages also had glaucomatous visual field defects. A disc haemorrhage is therefore a very specific sign of glaucoma. Other findings which cause a suspicion of glaucoma were found to be very unspecific and could, as defined here, not have been used to ascertain a diagnosis of manifest glaucoma.

In 13 out of 15 subjects with glaucomatous visual field defects an enlarged cup was noted at the initial examination. Simple ophthalmoscopic selection of suspicious cups was thus much, and significantly $P \leq 0.025$ (Fisher's exact test), more sensitive than any other method employed here to identify persons with an increased probability of manifest glaucoma. No other method (except automatic perimetry) could, as applied here, have been used to exclude the possibility of a manifest glaucoma with an acceptable degree of certainty.

Discussion

A type of non progressive low tension glaucoma characterized by an acute onset with sudden visual loss and therefore probably greatly overrepresented in self selected materials has been connected with systemic haemodynamic and vascular disorders (Chumbley & Brubaker 1976). In the present more representative material glaucomatous visual field defects were not combined with reduced central visual acuity and not associated with diabetes, vascular diseases, haemodynamic crises, antihypertensive medication or aberrant systolic blood pressures. Almost every person deceased after the survey had reported such disorders or medications and it is conceivable – though not likely – that a high mortality could have masked a high incidence of glaucoma in persons with a short life-expectancy. Anyhow our finding that the prevalence of manifest glaucoma was unaffected by systemic haemodynamic and vascular disorders retains its significance in the present context.

Slit lamp examination may be compared with tonometry as a method for the detection of the glaucomas most likely to benefit from current antiglaucoma therapy, i.e. glaucomas with visibly obstructed aqueous outflow and very high intraocular pressure. The present study does not allow of any statements concerning the relative sensitivities, but the rate of persons with anterior segment pathology without visual field defects, i.e. "false slit lamp positives" was surprisingly high (4.5%). Tonometry was in fact the more specific method provided that the upper limit of "normal" pressure was raised to 21.5 mmHg.

In the present district a majority of patients subjected to antiglaucoma treatment were glaucoma suspects without known glaucomatous visual field defects. Congenital, acute and secondary glaucomas with glaucomatous visual field defects seldom escaped discovery but persons with similar defects caused by chronic primary glaucomas often remained untreated (Bengtsson *in press*). Many of the "positive" family histories were therefore accepted on inadequate grounds and the specificity might have been improved, e.g. by an inquiry into the records of untreated relatives, if the sensitivity of the family history had not been too low. A warrant such an effort. Approaching changes in the diagnosis and therapy of glaucoma may call for an early re-evaluation of this point.

Any improvement in sensitivity of tonometry can be obtained at will simply by lowering the upper limit of "normal" pressure but only at the cost of further impairments in specificity. In the present material, for instance, tonometry would have been as sensitive as ophthalmoscopy if persons with intraocular pressure higher than 18.5 mmHg had been considered not "normal" but only at the cost of considering 281 persons, i.e. 19% of all subjects to be ocular hypertensives.

Similarly, improvement in the specificity of cup inspection is possible by the use of more strict criteria. A notching of the rim or a large cup with steep edges extends

to the outer margin of the disc was noted in ten cases nine with and one without glaucomatous visual field defects. The sensitivity could have been restored almost completely by adding persons with disc haemorrhages. In fact a notching of the rim, a large cup with steep edges extending to the outer margin of the disc and/or a disc haemorrhage were noted at the initial examination in 16 persons, 12 with and four without glaucomatous visual field defects. In the three remaining subjects with glaucomatous visual field defects a notching of the rim (two cases) or an extension of a large cup with steep edges to the disc margin (one case) can be observed on the fundus photographs. It may therefore become possible to identify persons with manifest glaucoma by refined disc assessment with a reliability that approaches that of automatic perimetry (Bengtsson & Krakau 1979).

Disc haemorrhages are fleeting lasting from a few days to several weeks (Begg et al 1971). The observation in a cross sectional survey like the present one of disc haemorrhages in seven out of thirteen cases of simple glaucoma therefore implies not only that disc haemorrhages frequently recur but also that repeated examinations can be expected to reveal disc haemorrhages also in cases of manifest glaucoma simplex which happened to be free from haemorrhage at the initial examination.

The present study therefore seems to predicate that disc haemorrhages are not only a very specific sign of manifest glaucoma but also a sign common at one time or another to the vast majority of all persons suffering from simple glaucoma.

From a practical point of view this conclusion cannot change the fact that a disc haemorrhage is an elusive sign that often escapes observation.

From a theoretical point of view on the other hand the frequent occurrence of recurrent haemorrhages seems to be an important clue to the solution of many problems concerning the pathogenesis of glaucoma. The opinion of Drance et al (for references see Drance et al 1977) that the optic neuropathy in this group of patients is probably due to small vessel disease and that an imbalance between intraocular pressure and vascular pressure is not the only cause of visual field defects in glaucoma may be more universally valid than originally understood.

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COMPUTERIZED MERIDIAN PERIMETRY

A preliminary report

BY

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By a modification of the display of test points it is possible to use the computerized perimeter "Competor" for meridian testing. The test program gives estimates of the thresholds in two separate rounds, the second starting when the first is completed. Accordingly two profiles are plotted in the meridians tested. Furthermore a smoothed profile is drawn from the mean values of the two rounds. The outcome when testing normal subjects and cases with glaucomatous defects was studied and the stability of thresholds in normals and the decay in scotomatous areas with time is demonstrated.

Key words: computerized perimetry — static profiles — time dependence

Manual static perimetry is generally practiced by plotting the thresholds at points along a meridian passing through a scotomatous area. In its first version our automatic perimeter was provided with the lights placed along a meridian, but the number of test points were few and this apparatus merely served the purpose of studying the conditions precedent for computerized perimetry. In the present note we want to describe a version of the automatic perimeter "Competor" intended for plotting meridians in clinical cases.

The results of testing normal and pathological cases will also be touched upon.

Apparatus

The design and general appearance of the apparatus, as well as its electronic parts, are the same as previously described (Heijl & Krakau 1976) except that a number of

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It is possible by a simple change of the orders to the computer to determine the extension of the meridians tested and even to exclude one of the meridians from testing. In the present study the limit was put at ± 15 degree in both meridians. The time required for one session was 13–17 min, the mean of the normal group being 13.30 and that of the pathological group 14.45.

Both the normal group and in all glaucoma cases the meridians 45–225 degree and 135–315 degrees were tested.

The background illumination was 0.1 cd/m² for five patients treated with miotics and 1.0 cd/m² in all other cases.

Fig. 2 shows the outcome of two test sessions in a normal subject. The threshold values of the rounds after one and three changes of sign follow each other closely. At most points the difference is ≤ 1 step, exceptionally it amounts to two steps.

For comparisons between the outcome of different test sessions in the same subject a simple measure of performance (P) has proved valuable. It is the sum of

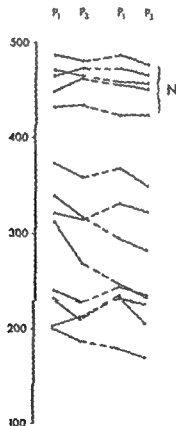


Fig. 3

P values from the first (P₁ and P₂) and second session (P₁ and P₂). N = normal group

the estimated threshold values of all points tested ($P = \sum_1^N x$) P is automatically calculated at the end of a session

The programme described produces two P values in each session one from the first change of sign (P_1) and one from the 3rd (P_3)

As seen in Fig 3 the P values show a small variation in the normal subjects. There is no significant trend towards lower values in P_3 than in P_1 as was shown by means of a simple sign test

In order to test the significance of the differences in performance between the various rounds we presume that the random variation of the threshold estimates is similar at all tested points. The series of differences between the thresholds of each point of two rounds to be compared is formed. The hypothesis that the differences of these paired observations do not on the average differ from zero was tested by means of the sign test. There was no significant difference (on the 5 per cent level) in any of the normal cases between the two rounds in the same session or between the same rounds in different sessions. The mean differences ($P_1 - P_3/60$ etc.) which are ≤ 0.22 thus fall inside the random variation of the measurements

As must be expected the differences of the dispersions in the mean value series are considerably smaller than in the primary series. In agreement with previous results (Holmin & Krakau 1979) it is concluded that the results are fairly stable at repeated testing of normal subjects

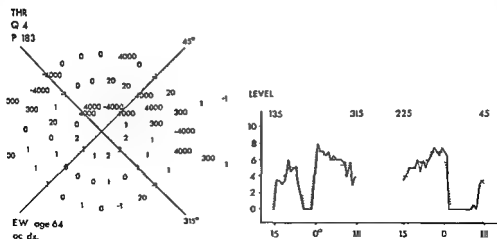


Fig 4

- A Visual field obtained with circular pattern system and threshold programme showing defects in the upper part (glaucoma spl)
 B Meridians (mean profiles) through the defects. First session fulldrawn second session dotted lines

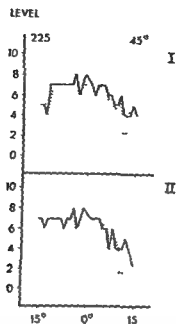


Fig. 5

Deepening of relative scotoma in a case of glaucoma spl from first (fulldrawn) to 2 (dotted) change of sign rounds. Note that the normal parts of the profiles remain practically unchanged. Similar pattern in both sessions (I and II).

In *pathological* cases meridians are chosen so as to cut through scotomata (Fig. 4). In the present group it occurred that the same meridians as those tested in the normal group were most appropriate. Accordingly, the P values are lower in the pathological group than in the normal one. The values are also often less stable and show in all sessions but one a tendency to decay with time (i.e. $P_2 < P_1$). This is in fact accordance with previous findings among glaucoma cases using long session test (Holmin & Krakau 1979). The dispersion of the point to point differences between the first and second sessions or between the two rounds in a session are as a rule higher than among the normal, which indicates that the change in performance is not merely a general change of level. This is also very clear at inspection of meridian plottings: the parts falling in scotomata are as a rule less stable than normal parts of the meridians (Fig. 5 and 6). The hypothesis of homoscedasticity of the tested points is therefore not tenable in scotomatous fields.

When the whole set of test lights has to be used it is likely that the program described may be found too time-consuming and a simpler version may be preferred. By a few orders in the language "Basic" it is easily achieved that the test process ends after the first change of sign. One may also ask if there are any reasons at all to go on testing until the third change of sign. The gain might be that a test

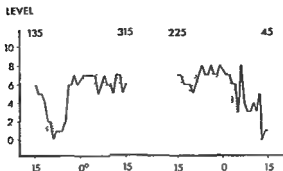


Fig 6

Scotoma increase in a case of glaucoma spl from first (fulldrawn) to second (dotted) session
Normal parts remain practically unchanged

curve from the first and the 3rd round can be formed and this smoothes random irregularities. Furthermore, when it comes to pathological cases, it may be a piece of valuable information that a defect is provoked or increased when the testing is extended in time.

We conclude that the system for automatic meridian perimetry described has a density of test points along the meridians which makes it well suited for the tracking of defects. For the follow-up of defects, good help is rendered by the performance measure P , which compresses information of a profile into a single number. Ordinary statistical tests are applicable to a series of such performance values, and the degree of significance in a suspected progress can be assessed.

Acknowledgement

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ULTRASONIC INVESTIGATION OF THE EYE WITH A NEW CONTACT METHOD AND WITH THREE DIFFERENT REAL TIME PRESENTATIONS

BY

ARVO OKSALA, MAURI LUUKKALA and PEKKA MERILÄINEN

The ultrasound examination was carried out by means of a crystal with a 40° sector scan which was pressed either against the eyelid or directly against the sclera. The real time display was obtained by either A mode, B mode or combined A/B mode scanning. In clinical applications both the resolution capacity and the sensitivity of the device were good. A mode scanning gave the greatest accuracy in amplitude measurement while the A/B mode yielded the most concrete topographic picture of the target.

Key words: ultrasonography -- contact method -- real time presentation

The use of ultrasound in ophthalmic diagnostics began during the 1950's (Mundt & Hughes 1956, Oksala & Lehtinen 1957, Baum & Greenwood 1958). The two first mentioned investigators who used A mode ultrasound published only one paper on the diagnostics of intraocular tumors. In the late 1950's and early 1960's Oksala & Lehtinen concentrated on the development of the A mode, Baum & Greenwood on that of the B mode. In the late 1960's and particularly during the 1970's the advantages and disadvantages of the two methods were studied in many research centers and their clinical usefulness was found to be greatest when they were used to complement one another.

One major practical problem in B mode examination has been the necessity of the water bath between the transducer and the eye. To eliminate this necessity Bronson developed (in 1967) the first hand held scanner for the B mode examina-

of the eye and orbit. In this procedure a case within which the transducer is usually pressed against the eyelid. During the 1970s this procedure has been increasingly applied because of its simplicity.

In the following paper we present a new examination device using a hand-held scanner which is pressed either against the eye ball or directly against the surface of the eye and which permits A- and B- and A/B mode examinations.

Methods

Fig. 1 shows the device. On the left at the top of the cable lies the transducer head. During the examination either stands still (A mode) or performs a sector-scan of 40° (B or A/B mode).

The hand-held scanner head of this device consists of the transducer, a DC motor and a mechanism to transform the rotational motion of the motor into a sector-scanning motion of the transducer. In addition a simple optical sensor is coupled to the motor axis to generate a signal to produce a synchronous sweep on the display tube. The scanning mechanism results in a sinusoidal movement of the transducer as a function of time. The scanning rate is variable. A rate of about 20 frames seems to be optimum when a pulse repetition rate of 3 kHz has been used.

The active element of the standard transducer is a focused 6 MHz piezoelectric PZT crystal with focal length of 24 mm and diameter of 6 mm. The crystal is backed and covered with impedance matching layers in the conventional manner. A typical bandwidth of 40% has been measured for the transducers. This means an axial resolution of about 0.5 mm which is in agreement with the experimental results. The maximum lateral resolution is obtained at the focal distance, i.e. in this case at the front of the retina and it has been found to be likewise 0.5 mm.

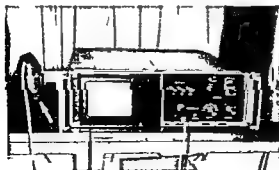


Fig. 1

The new ultrasound equipment. On the left an arrow indicates the transducer.

This ultrasonic ophthalmic scanner has three real time display modes. In addition to the conventional A and B mode there is a third one which we call the A/B mode. This mode displays a quasi three dimensional synthetic perspective image of the tissue under examination containing simultaneous information about the anatomical features of the *B* cross section and echo amplitudes with all the dynamic capabilities of the A mode. However part of the information is lost by the partial overlapping of the echo signals. A HP 133B display tube with 80 × 100 mm² frame size has been used as a display element so far but will be replaced by a HP 133A memory tube with variable persistence. The pulser amplifier electronics of the device follow standard solutions. An internal electronic caliper for the measurement of various distances in all three modes is included. The number of panel controls has been kept to a minimum. A more detailed description of the mechanical structure and electronics of this ultrasonic ophthalmic device has been published (Meriläinen & Luukkala 1970). The industrial production of the device will be started *Lasertek Oy*, Helsinki, Finland.

The eye and orbit of a patient is examined after anesthetization with a drop pressing the transducer either against the eyelid or the sclera. In an axial A scan the immovable transducer is pressed against the centre of the cornea while in an axial B mode or A/B mode scan the moving transducer is pressed against the eyelid and the sector scanning occurs through the lens. In the diagnosis of diseases of the eye and orbit past the lens A mode, B mode and A/B mode examinations are carried out chiefly by lightly pressing the transducer directly against the sclera or by pressing it against the eyelid and aiming the beam at the desired point in the eye or orbit.

Results

Echograms of a healthy eye

Fig. 2 shows echograms of a healthy eye resulting from A mode (Fig. 2a), B-mode (Fig. 2b) and A/B mode scanning (Fig. 2c). On the left is the transmitter impulse followed by an acoustically homogeneous space representing the vitreous body. On the right we see the echoes from the fundus of the eye and from the retrobulbar tissues. The vitreous body of the healthy eye of a young or middle aged subject is shown by modern techniques to be acoustically homogeneous. Fig. 3 shows the echograms of the degenerated vitreous body of an old person. In the A mode one or more echoes are reflected from the vitreous body and their location and amplitude vary rather quickly (Fig. 3a). In the B mode these echoes take the form of spots varying in number and location as well as a slight variation in luminance (Fig. 3b). In the A/B mode the degeneration of the vitreous body appears as a series of peaks of varying amplitude, location and number whose amplitude can be measured with an amplification control device equipped with a decibel scale (Fig. 3c).



Figure 1
 Angle of incidence of light rays from a young leafy eye. The rays are shown by a
 1. Angle of incidence of light rays from a young leafy eye. The rays are shown by a
 2. Angle of incidence of light rays from a young leafy eye. The rays are shown by a
 3. Angle of incidence of light rays from a young leafy eye. The rays are shown by a





Fig. 4

Echograms of retinal detachment by means of A mode (a) B mode (b) and A/B mode (c). An R indicates echoes from the retina and arrows echoes the vitreous degeneration. N shows the optic nerve



Echograms occurring in some eye diseases

The echograms of an idiopathic detachment of the retina are shown in Fig 4. An A mode scan (Fig 4a) shows that the echo from a detached retina is considerably higher than those reflected from the degeneration of the vitreous body. In B mode scanning (Fig 4b) the detached retina appears as a nearly continuous series of light points which in case of a central detachment is attached to the papilla. In A/B mode scanning (Fig 4c) the detached retina appears as a steep chain of peaks, the vitreous degeneration as a series of separate echo peaks (indicated by an arrow) and in this figure the relatively homogeneous subretinal fluid as an acoustically empty space.

The echograms of a choroidal melanoma are also highly characteristic. In A mode scanning (Fig 5a) relatively high and static echoes which reach the fundus are reflected from the area of the tumor. In B mode scanning (Fig 5b) the echoes from the tumor area are not as close together as in the A mode, but a simultaneous detachment of the retina is clearly observable. In A/B mode scanning (Fig 5c) the echoes reflected from the tumor are more frequent than in the B mode and their amplitude is measurable in decibels. A possible simultaneous detachment of the retina, if the beam hits it, is observable as a series of echoes, as seen in Fig 4c.

If much blood has penetrated into the jelly-like vitreous, A mode scanning (Fig 6a) shows numerous high echoes reflected from the vitreous space. Using the A/B mode (Fig 6b) the localization of the haemorrhage is shown in the sector examined and also the great variation in echo amplitude. In Figs 6a and b the echoes reflected from the haemorrhage are lower in the anterior part of the vitreous body than in the middle and posterior part.



Fig 6

Vitreous haemorrhage can reflect different kind of echoes, often with high amplitudes, as seen by A mode (a) and also over large area, as seen by A/B mode (b). It shows the haemorrhage.

Discussion

Compared with many earlier ultrasound devices used in ophthalmological examination the device presented offers certain clear advantages: the frequency of 6 MHz and the focusing give the device both good resolution capability and sufficient sensitivity. The latter means, among its other consequences, that the vitreous degeneration caused by aging and the acoustic heterogeneity of the subretinal space in cases of detachment of the retina are usually observable. The device also makes possible to diagnose diseases of the orbit up to about 2.5 cm from the rear eye wall. Various measurements of the eye and orbit can also be performed with an accuracy of ± 0.5 mm.

The possibility of performing an examination in A mode, B mode and A/B mode makes the quantitative and topographic analysis of the echograms relatively exact. The amplitudes of the echoes can be measured with a decibel scale more precisely with the A mode, but the same measurement is also possible with the A/B mode, which indicates topographic conditions more accurately. This kind of measurement is clearly more exact than when the grey scale alone is used. In our experience the A/B mode gives a more concrete picture of both the healthy and pathological eye than the B mode alone.

The examination is easy to carry out by pressing the transducer lightly either directly against the eye or against the lid. When the eye is examined through the sclera, the sector scanning causes a slight movement of the conjunctiva, which, however, has not been found to cause any harm to the eye. After routine clinical use of the procedure for almost two years, not even conjunctival sagitation has been observed.

The localization is more accurate if the transducer is pressed against the surface of the eye rather than against the lid, because the position of the beam inside the eye can then be better estimated. When the transducer moves across the surface of the eye, the topography can be described more precisely than with the immersion method, where a water column lies between the transducer and the eye. If the eye is in a water bath during the examination, we can also see the echograms from the anterior parts of the eye, such as the cornea and iris, but cases where such an ultrasound examination is really needed are very few.

The small size of the examination device and its easy handling make it suitable for use wherever eye diseases are diagnosed and treated. In the examination of the vitreous space, even when optical examination is possible, we often get information by means of ultrasound from a considerably larger area than would be possible optically. In vitrectomy, where the immersion method can not be used, this kind of ultrasound examination is easy and aseptic.

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ABSORBABLE SUTURES (DEXON AND VICRYL) IN THE CORNEOLIMBAL INCISION

Used in lens implantation surgery

BY

NIELS VESTI NIELSEN JENS CARL HØJBJERG and ERIK WESTERLUND

In 115 consecutive cataract extractions with implantation of artificial lens a clinical evaluation of absorbable sutures polyglactin (910) (Vicryl® 7-0) and polyglycolic acid (Dexon® 8-0) – in corneolimbic incision has been performed. The corneolimbic wounds were closed by continuous suture technique with one double loop knot at the 12 o'clock and 5-6 loops on each side of the 12 o'clock knot. The suture had disappeared after 8 weeks in 95% of the eyes. Two months after operation the visual acuity, the power of corneal astigmatism and astigmatic orientation remained unchanged in both the Vicryl® and the Dexon® sutured group. This provided effectuation of early full prescription of glasses.

Most complications in this material were suture independent and appeared during the early postoperative period. In only one eye inadequate wound closure was noticed. Shallow anterior chamber and hypotonia of short duration occurred in 3 eyes. Four of these patients developed corneal dystrophy. In the Vicryl® sutured group suture reactions took place in 87% of the eyes. With dexon® suture no such reaction appeared. The use of absorbable sutures in corneolimbic incision technique implies several surgical advantages and is seemingly safe.

Key words: lens implantation surgery – absorbable sutures (Dexon® 8-0 Vicryl® 7-0) – corneolimbic incision – corneal astigmatism – complications

In recent years the advantages of the synthetic absorbable sutures polyglactin 910 (Vicryl®) and polyglycolic acid (Dexon®) in ophthalmic surgery have been demonstrated in several studies (Bartholomew et al 1976 Blaydes 1979 Dunlap et al 1976 Furguele 1974 Sherman 1979 White & Parks 1974 Williamson 1974) however Klemetti (1979) recently has reported frequent complications using Dexon® 7/0 in corneoscleral incision of cataract operations.

At the present the properties of these sutures in corneal or corneolimbic surgery have not been reported.

The aim of this study has been to estimate the efficacy and safety of resorbable sutures in corneolimbic incisions performed in lens implantation surgery.

The reasons for the application of this surgical technique in our department have been the requirements of obtaining an improved operating field during implantation of clip lenses by avoiding a conjunctival flap reducing the frequency of haemorrhages during surgery rendering the late removal of permanent sutures superfluous and providing an early prescription of definitive glasses post-operatively.

Material and Methods

The material comprised 110 consecutive intracapsular cataract cryoextractions with implantation of an iris clip lens (Federow modification of iris clip lens) in 100 patients.

Vicryl® sutures were used in 60 eyes of 50 patients (29 women and 21 men) with a mean age of 76 years (range 61–91 years) from June 1977 to April 1978. Thereafter Dexon® sutures were applied in the following 50 eyes of 50 patients (19 women and 31 men) with a mean age of 74 years (range 64–89 years) until December 1978. All patients had a senile cataract. Patients with corneal dystrophy shallow chamber significant iris atrophy uveitis glaucoma diabetes mellitus and retinal vascular occlusive disorders were excluded from lens implant surgery.

In 80 cases the operation was performed by the chief surgeon and in the remaining 30 cases by the second surgeon.

Preoperatively 500 mg acetazolamide was administered intravenously in order to lower the intraocular pressure. All operations were performed in local anaesthesia. Both surgeons used operating spectacles with a 2 × magnification.

The surgical technique was unchanged in this material. A corneolimbic incision in front of the limbal blood vessels with an extent of 180° was made with a preplaced suture localized at 12 o'clock. A perforating incision about 5 mm in extent to the anterior chamber firstly was performed with a knife (razor blade). Thereafter the incision was widened with a corneal scissor perpendicular to the corneal surface. Two iridectomies at the 10 and 14 o'clock position were made.

The wound was closed by continuous sutures (Vicryl® 7/0 or Dexon® 8/0) with 5–6 loops on both sides of the 12 o'clock position where a double knot was primarily tied (Fig. 1). Finally the continuous sutures were tied with an unburned knot at the 9 and 3 o'clock positions.

Postoperatively the patient was treated with prednisolone eye drops 3 times daily for 2 months and pilocarpine 2% eye drops for half an year 2 times daily.



Fig 1

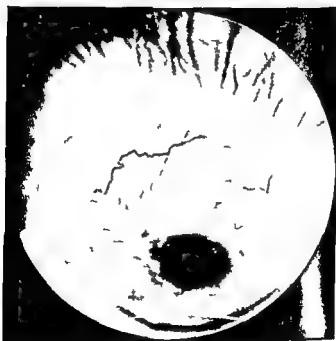


Fig 1b

Figure 1a and b

A corneolimbic wound continuously sutured with Dexon® 8-0. In 10 d a vascular pannus has developed from the limbal region (Fig 1a). The sutures have disappeared after 4 weeks (Fig 1b).

before cataract operation corrected visual acuity slitlamp examination intraocular pressure by Goldmann's applanation tonometry and ophthalmoscopy were registered. Postoperatively the patients were controlled daily in the first 10 days and regularly 1 month and 2 months after operation. A final reexamination took place after 6 months in the Dexon® sutured group and 15 months in the Vicryl® sutured group. On these controls corrected visual acuity corneal astigmatism and axis by Javal Schiotz keratometry and intraocular pressure were recorded. Rupture and disappearance of sutures and any complications after operation were evaluated by slitlamp examination and ophthalmoscopy.

Statistical Methods

The results were evaluated by using chi-square test and Mann-Whitney rank sum test.

Results

Visual acuity corneal astigmatism and intraocular pressure

It appears from Table I that a definite improvement of corrected visual acuity occurred already after 2 months ($P < 0.001$). At follow up examination no significant change in final corrected visual acuity was observed compared to the results obtained at 2 months of observation ($P < 0.10$). A final visual acuity $\leq 6/9$ (67%) was obtained in 70% of the patients (72% in the Vicryl operated and 68% in the Dexon operated group).

Concerning postoperative examinations of corneal astigmatism and the axial orientation (Tables II and III) it is noted that no significant changes of these values were appearing at the 2 months control ($P < 0.10$). In both groups the final orientation of the axial orientation of corneal astigmatism was against the rule. Vicryl® 87.6° and Dexon® 82.1°.

Table I

Visual acuity pre- and postoperative in 60 Vicryl® and 55 Dexon® sutured eyes. No further improvement of visual acuity is occurring on follow up examinations ($P < 0.01$).

		Pre-op	10 days	1 month	2 months	follow up
Visual acuity mean (\pm sd)	Vicryl	0.15 (0.17)	0.29 (0.18)	0.46 (0.27)	0.67 (0.29)	0.70 (0.30)
	Dexon	0.23 (0.22)	0.37 (0.29)	0.53 (0.28)	0.69 (0.30)	0.73 (0.31)

Table II

Postoperative power of corneal astigmatism in 60 Vicryl® and 50 Dexon® sutured eyes. Astigmatism is insignificantly changed after 2 months ($P < 0.01$)

		10 days	1 month	2 months	Follow-up
Corneal astigmatism	Vicryl	4.12 (1.60)	3.80 (1.48)	3.52 (1.41)	3.1 (1.31)
mean (\pm SD)	Dexon	3.80 (2.34)	3.60 (1.60)	2.91 (1.40)	2.80 (1.50)
ΔK (diopt)					

Cicatrices and disappearance of sutures

Repeated inspections of the cicatrices disclosed no inadequate wound healing except in one case (Table IV). In Fig. 1 the wound and continuous suture in corneolimbus incision is illustrated 10 days and 8 weeks after operation. After weeks the sutures (both Vicryl® and Dexon®) had ruptured and were disappeared in 95% of the patients. In none this occurred before 4 weeks. In all eyes we saw various ingrowth of a small vascularized pannus from the limbal region to the cicatrice. This zone of fibrovascular tissue persisted until the sutures disappeared. In 40 patients (46 eyes) sutured with Vicryl® an annoying foreign body sensation with various hyperaemia and mucus secretion occurred. Dexon® sutures did not cause such a reaction.

In no patient was the development of pathological corneal excavations at the corneolimbus cicatrice observed.

Table III

Postoperative axial orientation of corneal astigmatism in 60 Vicryl® and 50 Dexon® sutured eyes. Already after 10 days the astigmatism in the Dexon® group is orientated against the rule. One to two months after operation no further significant shift of the axial orientation is noted ($P < 0.10$).

		10 days	1 month	2 months	Follow-up
Axial orientation of corneal astigmatism	Vicryl	62.9° (44.8)	81.4 (32.5)	84.0° (21.5)	85.6° (14.5)
mean (\pm SD)	Dexon	79.6° (45.2)	79.5 (33.9)	80.5 (18.4)	80.1 (12.4)

Table IV

Postoperative complications in 60 Vicryl® and 55 Dexon® operated eyes. The number of eyes with late presence of shallow anterior chamber hypotonia and corneal dystrophy (right column) have continued complicated from the early postoperative period. No significant difference of complications between the Vicryl® and Dexon® group was disclosed ($P < 0.10$)

Complications (number of eyes)	Suture					
	Vicryl			Dexon		
	During surgery	Early ≤ 10 days	Late > 10 days	During surgery	Early ≤ 10 days	Late > 10 days
Hypaema	2	4		1	1	
Vitreous loss				0		
Dislocation of lens implant		1		1	2	
Inadequate wound closure					1	
Shallow anterior chamber		2			3	1
Hypotonia (< 9 mmHg)		8			11	1
Hypertension (> 22 mmHg)			4			3
Uveitis		1			3	
Pupillary exudate membrane						1
Corneal dystrophy		2	2		2	2
Macular oedema			1			0

Complications

Early and late complications are listed in Table IV. It is noted that both shallow anterior chamber and ocular hypotonia with cornea lens implant contact appeared in 5 patients with a duration of 1 to 2 days. In these cases no obvious leakage of aqueous humor from the cicatrice was disclosed by Seidel's test. Inadequate wound closure appeared in one eye because of sutures placed too superficially which in few days cut through the corneolimbic tissue. A sufficient closure was obtained by resuturing the eye.

Discussion

The results of this study indicate that the resorbable sutures polyglycolic (Dexon® 8/0) and polyglactin 910 (Vicryl® 7/0) are useful for suturing corneal incision in lens implant surgery.

The main difference between the applied sutures was the appearance of suture complaints in the Vicryl® sutured group.

Considering the healing of the corneolimbic wound we noted an early form of a superficial vascularized granulation tissue from the vessel arcades of iris. Presumably this accelerates the corneal healing and provides the efficacious use of absorbable sutures in the corneolimbic region.

In experiment on the rabbit cornea it has been demonstrated that corneal wounds at limbus heal faster than more central placed corneal wounds (Gas Dohlman 1968). Thus a significant higher tensile strength of the peripheral corneal wounds in rabbits was obtained already after 12 days. It is possible that the absorbable sutures used in our series further stimulate the formation of fibrovascular tissue in the corneolimbic wound. Flaxel & Swan (1969) have studied the limbal wound healing histologically in human aphacic eyes. They observed a remodelling of the corneal wound first took place after 46 days. According to the time of resorption of the sutures in the present series as in other studies (Dund et al 1976, Bartholomew et al 1976, Furgule 1974) coincides with both the time of high corneal tensile strength and the remodelling of the wound (Gasset & Dohlman 1969, Flaxel & Swan 1969). However Condon & Hill (1973) have in experimental animal studies shown that a critical and direct suture dependent phase of corneal wound healing is present during the first 6 days after incision. Vicryl® 11/0 sutures used in central corneal wounds in rabbit obviously remain intact for only 13 days (Faulborn & Theopold 1977). It must however be assumed that only minor absorption and decrease in tensile strength of Dexon® 8/0 and Vicryl® 7/0 corneal sutures are appearing in this early phase (Craig et al 1977). However further studies of the tensile strength of Dexon® and Vicryl® in corneal wounds are necessary. It is remarkable that no rupture of Dexon® 8/0 or Vicryl® 7/0 suture in our material occurred within the first month after operation.

Concerning the power of corneal astigmatism and the axial orientation it is noteworthy that no significant changes occurred 2 months after operation with Dexon® and Vicryl®. This practically implies that early full prescription of astigmatism can be performed contrary to suture techniques with a non resorbable suture like nylon 10/0 (Thygesen et al 1979). However the power of the final astigmatism in our material appeared to be about 1 Diopter higher (Thygesen et al 1979).

In Klemm's series (1979) a notable high frequency of suture dependent complications appeared after suturing corneoscleral wounds with Dexon®.

these frequent complications however have not been demonstrated by others (Bartholomew et al 1976; Blaydes 1979; Furguele 1974; Sherman 1979; White & Parks 1974; Williamson 1974). Moreover the majority of the complications in Jemetti's material (1979) occurred 22-42 days after operation. In our material most complications probably were suture independent and appeared during the early postoperative course (≤ 10 days).

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HISTOPATHOLOGY OF CLINICALLY SUCCESSFUL INTRAOCULAR LENS IMPLANT

BY

ULF KRAUSE and AHTI TARKKANEN

A 50 year-old man had an intracapsular cataract extraction with placement of a Worst medallion iris supported lens. After 6 days postoperatively the eye appeared quiet, the cornea was clear and the implant in place. The patient died 17 days postoperatively of subarachnoid haemorrhage. Histopathological examination revealed slight loss of endothelial cells in the upper corneal region, pressure atrophy without signs of repair of the iris stroma, loss of pigment epithelial cells of the iris in places as well as local pigment epithelial cell proliferations. The chamber angle was open and the anterior uvea revealed only a minimal inflammatory cell reaction. The unoperated opposite eye showed cortical cataract as well as advanced hypertensive retinopathy.

Keywords: lens implant - histopathology - cataract extraction

In the recent surveys of intraocular lens implant surgery promising visual results have been reported (Jaffe 1976). The clinical complications are also reflected in the histopathological reports which have been compiled in the Table I. The earlier reports deal with the posterior chamber implants by Ridley and the different types of anterior chamber implants. The recent reports deal with present iris supported or indo-capsular type implants. As, however, we have been unable to find a report of a clinically successful implant procedure with a short observation period, we thought it justified to report our observations on a clinically successful case of implantation of the Worst medallion lens where the patient unexpectedly died 17 days postoperatively.

Table 1

Some histopathological studies on eyes with intraocular lens implant

Author(s)		Postop time	Type of lens implant	Comments
Reobald	1953			
Intelen & Subermann	1956	1 year	Posterior chamber lens of Ridley	post mortem
Rancos et al	1956	1 1/2 - 20 months		
Smith	1956		Ridley	
Shotton & Choyce	1959	6 weeks	Anterior chamber implant (Strampelli)	post mortem
Binkhorst	1959		Anterior chamber implant	post mortem
Shotton & Boberg Ans	1961	1/2 year	Anterior chamber implant (Boberg Ans)	post mortem
Iresnick	1969		Anterior and posterior chamber implants	17 enucleated eyes 11 postmortal eyes
Mauman & Ortbauer	1970	8 years	Dannheim	post mortem
Van Schot	1974	7 weeks - 5 years	Binkhorst	2 enucleated eyes 8 postmortal eyes
Alfve	1976		Copeland	post mortem
Fosier et al	1977	17 weeks	Binkhorst	Fungal endophthalmitis

Material

A 50-year-old man had a kidney transplant due to chronic glomerulonephritis in 1974. The postoperative regime consisted of systemic corticosteroid and antihypertensive medication. The patient noticed a decrease of vision in 1975. In 1977 he had difficulties in reading and was unable to drive a car. A dense posterior cortical opacity was present in both lenses as well as signs of a hypertensive retinopathy. The patient had an intracapsular cataract extraction in the left eye in August 1977 with placement of a Worst Medallion iris-supported lens. A Graefe section with enlargement of the operative wound with scissors was performed. The artificial lens was implanted with some difficulty as the anterior chamber remained shallow due to vitreous bulge. The operative wound was closed with 10/0 nylon monofilament sutures. The lens was sutured to the iris with one perlon suture. The anterior chamber was reformed with Balanced Salt Solution® (Alcon). During the postoperative period the lens remained in place, the cornea was oedematous for six days. After that the eye when observed with a hand held bin microscope was quiet, the cornea clear and the lens without motion in place. The optic disc appeared engorged with retinal haemorrhages.

After the first postoperative day the patient went comatose and a subarachnoid haemorrhage due to a hypertensive crisis was diagnosed. The patient seemed to recover but suddenly 17 days postoperatively. At autopsy a rupture of the basilar artery aneurysm subarachnoid haemorrhage were diagnosed. Both eyes were obtained at autopsy.

Results

Right eye

Macroscopic and microscopic examination of this unoperated eye showed corneal cataract and advanced hypertensive retinopathy.

Left eye

Macroscopic examination. The eye was opened through the vertical meridian showed no lens remnants. The implant was in place with no reaction around it. The vitreous was clear. A few haemorrhages were observed in the retina. Haematoxylin-eosin, van Gieson, Periodic and Schiff and iron stains were prepared.



Fig. 1A

The cornea shows a well healed cataract section with posterior gap. Haematoxylin-eosin, $\times 30$.

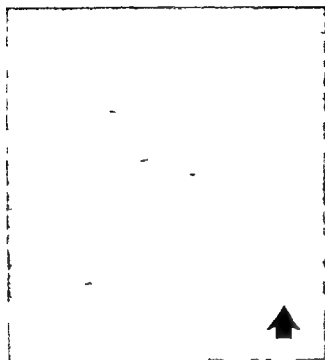


Fig 1B

especially the central side of the corneal wound shows marked loss of endothelial cells (arrow) Haematoxylin-eosin $\times 100$

Microscopic examination A well healed corneal cataract section with some posterior pigmentation is present (Fig 1A). The upper region of the cornea shows also some loss of endothelial cells (Figs 1A B). The chamber angle is open and the trabecular meshwork is loose. The iris stroma is moderately atrophic with thinning in the pupillary region and stromal atrophy without signs of repair in the mid periphery (Figs 2 and 3). The pigment epithelial changes vary from atrophy to local proliferation and even cystic changes. Only a few lymphocytes and macrophages could be found in the iris. The ciliary body shows no pathological changes.

Discussion

Following intraocular lens implantation there is rapid endothelial cell loss (Bourne and Kaufman 1976). Such loss is permanent due to limited endothelial cell regeneration. A loss of endothelial cells is present also in our case although clinically the



Fig 2

There is pressure atrophy of the iris in the pupillary region as well as a pigment epithelial cyst. Haematoxylin and eosin $\times 60$

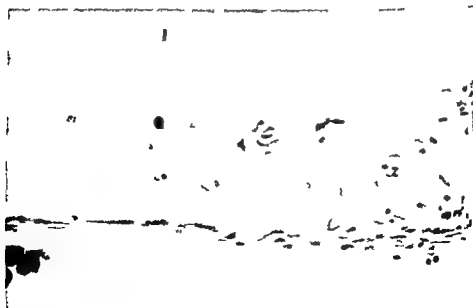


Fig 3

The iris shows marked atrophy of the stroma in the mid periphery as well as proliferation of the pigment epithelium. Haematoxylin and eosin $\times 60$ (i.e. implant)

nea remained clear. It is due to mechanical trauma during insertion which in the present case was made with difficulty due to vitreous bulge. The pressure atrophy of the iris stroma in our case confirms the observations of Manschot (1974). Its presence already 17 days after operation is however noteworthy. The changes of the pigment epithelium were similar to those described by Jaffe (1976) and Manschot (1974). Atrophy and proliferation due to surgical trauma and mechanical pressure and friction by wire fixation loops.

The atrophy is a momentary mechanical process as one is able to note marked pigment dispersion during the insertion maneuver.

According to Manschot (1974) of the 2724 anterior chamber lenses of the Binkhorst type implanted before 1972 in the Netherlands only one eye had to be excised because of postoperative complications. Hoffer (1978) reports the removal of 33 out of 70 000 eyes (0.05%) containing implants.

In the present case the implant was well tolerated indeed. In spite of the loss of endothelial cells there were clinically no signs of corneal decompensation. The iris changes may in part explain the reported cases of luxation or subluxation of the implant by the erosion of the loops through the atrophic iris. Still in our case the implant was well in place. As the long term results of implant surgery are uncertain (Jaffe 1978) collection of clinical and histopathological data should be encouraged.

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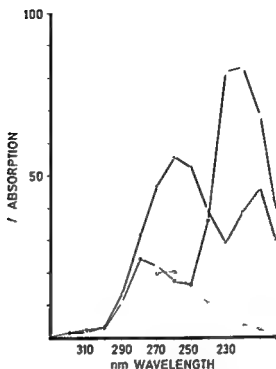


Fig 2

The curves illustrate an estimate of UV ray absorption by different potential substances in the cornea epithelium. The ascorbic acid concentration (white circles) corresponds to the amount of the rabbit cornea epithelium, whereas the protein (circles) and the nucleic acid (triangles) correspond to the concentrations in liver cell.

endothelium may be impaired during an acute attack of photophthalmia. Even moderate cell loss may by recurring attacks be a factor contributing to decompensation of the cornea. This possibility should be tested in separate experiments by looking for a change in the cell number after UV ray exposure.

As pointed out by Cogan & Kinsey (1946) the effects of UV radiation on the cornea is closely related to problems concerning the development of solar erythema of the skin, and both of these reactions are again aspects of the general problem how radiation may influence biological activity. A key role in the involved reactions is played by target molecules transforming the physical energy into biochemical processes of the tissue, and a wide variety of substances (proteins, nucleic acids, amino acids) have been taken into consideration as potential receptors. Surprisingly, the AA has not been included among these components, although this substance is a very labile one with high molar absorptivity (Karavannis et al 1970). In order to get a rough estimation of the UV ray absorption of the AA compared

it of the proteins and the nucleic acids in the rabbit cornea epithelium all these substances were run separately. The AA content of these cells is 118 mg/100 g (Pitts 1976) and since estimates of the nucleic acid concentration of the rabbit cornea epithelium apparently are lacking the present experiment was run with values from liver cells i.e. 10% protein and 1.1% nucleic acid (de Roberts et al. 1965). Of course it is a rough approximation when the whole intracellular protein fraction is presented by rabbit serum albumin. It should also be kept in mind that the high protein synthesis of the liver cells implies that these cells are particularly rich in nucleic acids. This view is reflected by the observation that nucleic acids make up 3% of the dry weight in the calf cornea epithelium (Zigman et al. 1963) which would mean 4.4 mg instead of 11 mg in Fig. 2 when water is set to 80% of the cell. Thus the amount of nucleic acid used in Fig. 2 represents the definite maximum value and most probably this compound is considerably overestimated compared to AA and protein. In conclusion therefore it seems reasonable to include AA among the potential receptor molecules for UV rays in the cornea epithelium when wavelengths below 290 nm are involved.

Since the AA absorbs maximally around 265 nm at physiologic pH (Karayannis et al. 1977) it may be that the peak at 270 nm in the cornea threshold curve of Pitts (1976) partly refers to this substance. However the problem as to whether a component acts as a target substance is certainly not only a question of its molar absorptivity. The absorption of light energy and its ability to harm living tissue is so supposed to depend on the local concentration of the single substance within the cell and on the stability of the biochemical system in which it is participant.

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AQUEOUS HUMOUR AND ULTRAVIOLET RADIATION

BY

AMUND RINGVOLD

Studies on the ultraviolet ray absorption in the aqueous humour of rabbit, cat, monkey, guinea pig and rat showed marked species differences. In the rabbit aqueous the ascorbic acid, the proteins and some amino acids (tyrosine, phenylalanine, cystine and tryptophane) are together responsible for the total absorption, and a very great part of it refers to the ascorbic acid content. Accordingly species with significant amounts of ascorbic acid in the aqueous (monkey, rabbit, guinea pig) have a greater absorption capacity towards ultraviolet radiation than species (cat, rat) lacking this substance. This effect of the ascorbic acid may contribute in protecting the lens against the most biotoxic ultraviolet rays. It seems that the ascorbic acid concentration is highest in the aqueous of typical day animals and lowest in species being active in the dark, indicating a correlation between the aqueous ascorbic acid level and the quantity of incident light on the eye. The possible significance of changed aqueous ultraviolet ray absorption in the pathogenesis of human cataract development is discussed.

Key words: eye lens - aqueous humour - cataract - ultraviolet radiation - ascorbic acid

In the past several important reports dealing with ultraviolet ray (UV ray) absorption in the light pathway of the eye have shown that radiation of potential danger for the retina is largely cut out through filtering properties of the media (Widmark 1892, Marun 1912, Verhoeff & Bell 1916, Kinsey 1948). More precisely it was found that the lens roughly absorbs rays below 400 nm wavelength (Norren & Vos 1974), whereas the cornea and the aqueous humour remove most radiation shorter than 300 nm wavelength (Kinsey 1948, Bachem 1956, Hemmingsen & Douglas

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1970 Ringvold 1979) Accordingly the lens also has a physiologic protection against shortwave rays and it seems important to identify the different components involved in this mechanism as well as to estimate to what extent each substance contributes to this. As demonstrated in a previous analysis of the UV ray absorption of tissue specimens is rather complex (Ringvold 1979) the situation turned out to be much simpler in the aqueous humour and this is described in the present paper.

Material and Methods

Different species were used in this study: 6 rabbits (5 albino, 1 black), 5 domestic monkeys (*Cercopithecus aethiops*), 10 albino guinea pigs (250–300 g) and 18 albino (150–200 g). Apart from the cats, which were adult individuals of unknown age, the others were young adult individuals. The guinea pigs received 1 mg ascorbic acid (AA) per ml in the water, and the monkeys had a fruit consumption of one orange daily, where other animals received no additional AA supplies beyond that contained in their



Fig. 1

Absorption of ultraviolet radiation in aqueous humour from rabbit (black circles) and monkey (white circles). Each curve represents mean values from 6 eyes, and as seen in many other experiments the standard deviation does not exceed the markings printed.

od Apart from the rats which were anaesthetized with ether all animals were given pentobarbitone sodium intravenously (rabbits monkeys) or intraperitoneally (cats guinea pigs). In addition the monkeys were initially given 20 mg phenylcyclidine hydrochloride (ernylan Park Davis) intramuscularly. Blood and aqueous humour were rapidly aspirated from the left ventricle and the peripheral part of the anterior chamber respectively. In rabbits cats and monkeys the investigations were carried out on specimens from each single animal whereby aqueous from both eyes was collected as one volume. In order to obtain adequate volumes from guinea pigs and rats each specimen of blood and aqueous represented a collection from several animals: two groups each of 5 guinea pigs and two groups of 8/10 rats were investigated. After aspiration the AA content of both serum and aqueous was estimated by the method of Michaelsson & Michaelsson (1967). The aqueous specimens were tested by adding 50 μ l to 900 μ l serum both for sample and blank whereafter the procedure for serum analysis was followed. All investigations were run immediately after the samples had been aspirated. The spectrophotometric studies were performed with a Beckman DB G instrument using quartz cells with 2 mm light pathway and the specimens were all compared to distilled water. After spectrophotometry of the aspirated aqueous and

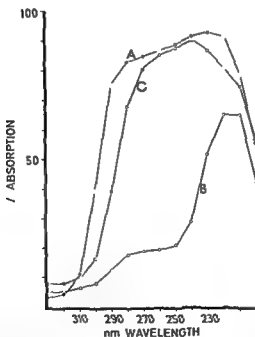


Fig 2

(Rabbit) Curve A Absorption of ultraviolet radiation through aspirated aqueous humour from one animal. Curve B Same sample after dialysis against distilled water. Curve C Same sample after a second dialysis against distilled water containing 25 mg AA/100 ml which was the aqueous level of this particular animal. Note the excessive AA concentration contributes considerably from the 240 nm limit towards higher wavelengths in raising the curve.

subsequent determination of its AA concentration the single specimens were dialysed in 1 l distilled water at +4 C overnight while continuously stirring to eliminate the molecular components such as AA and amino acids. Spectrophotometry of these showed the UV ray absorption referring to non-dialysable components of the aqueous (protein). In order to estimate the amount of UV ray absorption by the different dialysable components the non dialysable fraction was again dialysed against the initial AA concentration as a 2 l distilled water solution at room temperature for 4 h. The difference of the from aspirated aqueous and the spectrophotometry curve of the bag after this procedure illustrates the non AA part of the dialysable aqueous components. This set up has been used for all samples but since rats did not concentrate AA into the aqueous at all a second dialysis of these specimens was not carried out.

An artificial aqueous humour was made by mixing the following components in distilled water. The AA concentration was taken as in Fig 2 (25 mg/100 ml). Total protein (according to Neufeld et al 1972) was added as rabbit albumin (65 mg/100 ml) which in this context represents the different protein fractions of normal aqueous. The amino acids (tyrosine 1 mg/l, phenylalanine 20 mg/l, cystine 7 mg/l, tryptophane 5 mg/l) are amounts according to previous findings in rabbit (Reddy et al 1961).

Chemicals: l ascorbic acid, l cystine, l phenylalanine, l tryptophane, l tyrosine were Sigma products. In addition crystallized rabbit albumin (Armour) was used.

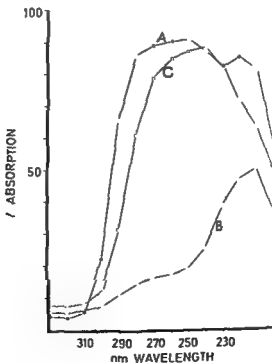


Fig. 3

(Monkey). Curve A: Absorption of ultraviolet radiation through a piece of illuminated from an animal. Curve B: Same sample after dialysis against distilled water. Curve C: Same sample after a second dialysis against distilled water containing 18.8 mg AA/100 ml which is the aqueous level of this particular animal.

Results

The UV ray absorption of the aspirated aqueous from 3 rabbits and 3 cats is shown in Fig. 1 (Spectrophotometric studies of the cornea from the same animals have been published elsewhere Ringvold 1979)

Figs. 2-6 show the UV ray absorption of different components present in the aqueous humour of rabbit monkey guinea pig cat and rat respectively. Curve A represents spectrophotometry of aspirated aqueous. Curve B illustrates how much the proteins contribute to the total aqueous absorption. The difference between curve C and each of the curves B and A visualises the UV ray absorption of the AA respectively the non AA part of the dialysable components. The difference between the curves A and C is certainly too high since the samples were too small for pH adjustment before running curve C (see below). For some unknown reason curve C is higher than the curve for aspirated aqueous above 300 nm and this phenomenon is more pronounced in the cat than in the other species.

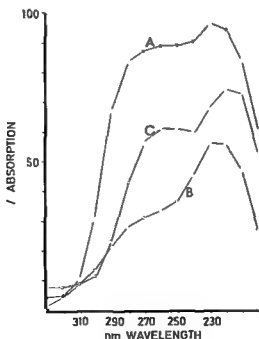


Fig. 4

(Guinea pig) Curve A Absorption of ultraviolet radiation through aspirated aqueous humour collected from 5 animals. Curve B Same sample after dialysis against distilled water. Curve C Same sample after a second dialysis against distilled water containing 9.6 mg AA/100 ml which was the aqueous level of this specimen.

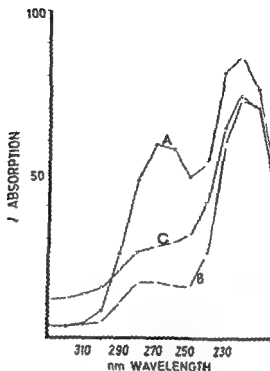


Fig 5

(Cat) Curve A Absorption of ultraviolet radiation through aspirated aqueous h collected from one animal Curve B Same sample after dialysis against distilled water C Same sample after a second dialysis against distilled water containing 1 mg AA l which was the aqueous level of this particular animal

In Fig 7 it is tried to imitate UV ray absorpuon of the aspirated rabbit aque mixing an artificial aqueous described under Material and Methods It is seen after adjustment to pH 7.0 with 0.1 M sodium bicarbonate the UV ray absor of this artificial aqueous roughly corresponds to that of the aspirated specimen.

In order to obtain the total *in vivo* UV ray absorpuon of the rabbit an aqueous the curves from Fig 1 are in Fig 8 adjusted to the real light path length through the anterior chamber of 3.5 and 4.5 mm respectively Discussion for further comments on this graph

The AA content for the aqueous humour from the different species *in vivo* mean \pm SD (mg/100 ml) were rabbit 23.5 (3.0) monkey 17.0 (2.5) guinea pig 4.2 cat 1.5 (0.4) rat 0.6 (0.5)

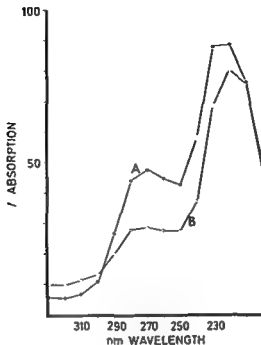


Fig 6

Rat) Curve A Absorption of ultraviolet radiation through aspirated aqueous humour collected from 10 animals. Curve B Same sample after dialysis against distilled water

Discussion

From this study it can be concluded that the rabbit aqueous humour does not contain any components contributing significantly in its UV ray absorption beyond AA serum protein and some amino acids. Conditions are probably similar in monkey and guinea pig in contrast to the aqueous of cat and rat in which the AA concentration is practically zero. Of particular interest is the considerable absorption of UV radiation by the AA which has also been mentioned by Balazs (1954). In view of the increasing evidence that UV radiation may cause cataract (Duke Elder 1926, Pirie 1968, Heyningen 1973, Kurzel & Wolbarsht 1973, Zigman et al 1973, Weiter & Finch 1975, Borkman & Lerman 1977, Hiller et al 1977) it is important to know that only wavelengths down to about 290 nm penetrate the earth (Robinson 1966). Furthermore, since light energy is inverse to its wavelength, the rays just above the 290 nm limit are likely to be the most biotoxic of the solar spectrum. It has previously been shown that as much as 20% of this radiation penetrates the rabbit cornea and the percentage is even higher in other species.

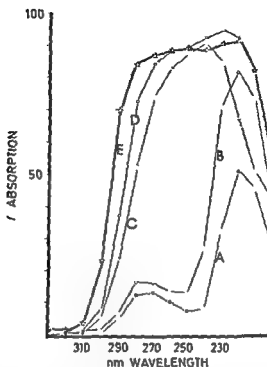


Fig 7

Absorption of ultraviolet radiation through an artificial aqueous humour (curve E). Curves A, B, and C show the absorption of distilled water containing amino acid, protein, and ascorbic acid, respectively. Curve D represents the components from A, B, and C as a sample showing pH 7.7. Curve F shows the solution from curve D after adjustment of pH 7.0. Broken line illustrates aspirated rabbit aqueous absorption (from Fig. 3 for comparison).

(Ringvold 1979). The fate of UV radiation penetrating the cornea certainly depends on the absorption properties behind this level, i.e. in the first place on the absorbing capacity of the aqueous humour. In Fig. 7 this capacity is shown for the rabbit eye, which is used as experimental model. Interestingly, Bachem (1967) reported that the UV action spectrum for the development of rabbit cataract is very characteristic: "This representative curve begins abruptly between 293 and 297 nm, reaches its peak near 297 nm, falls rather abruptly to 313 nm and has a long tail through the near ultraviolet." These observations are confirmed by Pitts (1977), which are interposed in Fig. 8. From this figure it is seen that the cataractogenic effect steeply increases from 310 to 300 nm, although the corneal transparency decreases in the same interval. From 300 to 290 nm, however, the decrease in transparency levels off; the expected further increase in the cataract curve is exchanged with an abrupt fall towards zero. It seems likely to connect the

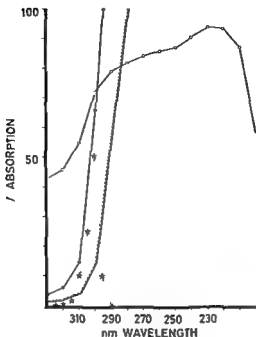


Fig 8

The absorption curves for aqueous humour from Fig 1 are adjusted to anterior chamber depth of 3.5 mm for the rabbit (black circles) and 4.5 mm for the cat (double line). Pictorial exposure threshold data for the action spectrum producing cataracts are shown (broken line with stars). The maximum value of this curve which corresponds to the lowest threshold value is arbitrarily set to the 50% level. The absorption curve of the rabbit cornea (Ringold 1979) has open circles.

phenomenon with the UV ray absorption in the aqueous humour which accordingly in the rabbit to some degree acts as a fluid UV filter protecting the lens. As shown in this study, this effect of the rabbit aqueous largely refers to its high AA concentration.

The rabbit eye may also be an adequate model to estimate the possible significance of the aqueous AA level in the pathogenesis of human cataract since the anterior chamber depth is 3.5 mm both in rabbit (Tripathi 1974) and man (Lovengren 1950) and the aqueous AA level is roughly of the same order in both species. It is evident from Fig 8 that a decrease in AA concentration of the aqueous means a lower slope of its absorption curve which opens for the most biotoxic radiation to the lens. One interesting question is therefore whether the AA level in aqueous in cataractous eyes is reduced or not. In fact, several authors report that this is the case (Muller & Buschke 1934; Nakamura 1935; Gala & Melka 1936).

Purcell et al 1954) although not all reports are in keeping with this conclusion (de Bernardinis et al 1965). It should be reminded that the AA concentration both in the aqueous (Kinsey 1947) and the cornea (Reim et al 1978) depends on the plasma level and from figure 2 in Kinsey (1947) it is seen that even small changes in the plasma content induces large fluctuations in the aqueous level. Since the plasma AA level is reduced about 50% in geriatric subjects (Loh & Wilson 1973) the protection of the lens against UV radiation is suggested to decrease with increasing age. Thus keeping in mind that cataract development probably has a cumulative effect over years it seems that reduced AA concentration in the aqueous humour either because of low serum level or because of defect concentration mechanism in the ciliary body may be a factor of significant pathophysiological importance. As Muller & Buschke (1934) came to the same conclusion, it is assumed that AA is necessary in the lens metabolism to maintain transparency. In contrast to their view the here presented "optical function" of AA is based on the high molar absorptivity of this substance.

According to the present study there are marked species differences in the lens protecting capacity of the aqueous. Thus both in the cat and the rabbit the absorption is rather low through the 2 mm thick aqueous sample. The cat however has a light pathway through the anterior chamber of as much as 4.5 mm (Trop 1974) and the total absorption is therefore significant in vivo despite extremely low AA values (see Fig. 8). It is evident that despite the deeper anterior chamber in the cat (4.5 mm) compared to the rabbit (3.5 mm) the cat's lens has the lower degree of UV protection at the two above 290 nm, largely due to the lower AA level in the aqueous. As a night animal the cat perhaps has no need of this protection. An aspect speaking in favour of this view may be seen in Table I indicating a general trend relating aqueous AA level of the different species to the quantity of incident light. Accordingly typical day animals are found at the top of the table with high values whereas night animals are at the bottom. In between there are species like dog and pig whose predecessors (jackal, wild boar) in part are operating during day break. It is of particular interest that the primates (owl monkey and galago? monkey) which both operate in the dark have low AA values in the aqueous in contrast to day monkeys (macaca and cercopithecus).

The common feature of all structures interposed between the body surface and the sensory cells of the eye is to mediate light onto the receptors and all along this pathway light energy is absorbed in different tissues before photons are transformed to nerve impulses in the outer segments. Since free radicals and unpaired electrons are generated wherever molecules are broken up by some energy process such as e.g. attack of light energy (Commoner et al 1974, 1977; Herrick & Bass 1977; Pathak & Stratton 1969; Lukiewicz 1972) it is reasonable to assume that substantial amounts of such components are continuously formed in the eye and

acetic acid concentration (mg/100 ml) in the aqueous humor. This is expressed in
 ble is largely based on Davis (1976, Table 2) and is not a direct reflection of the
 value.

Species	Acetic acid concentration	Value
Rabbit	100	Mollusk (100)
	(100)	Mollusk (100)
	25	100 (100)
	100	100 (100)
Ox	100	Mollusk (100)
	100	100 (100)
	100	100 (100)
	100	100 (100)
Horse	100	Mollusk (100)
	100	100 (100)
	100	100 (100)
	100	100 (100)
Man	100	Mollusk (100)
	100	100 (100)
Monkey (macaca mulatta)	100	100 (100)
(cercopitheca)	100	100 (100)
Cuneipogon	100	100 (100)
	100	100 (100)
	100	100 (100)
	100	100 (100)
Sheep	100	100 (100)
	100	100 (100)
Pig	100	100 (100)
	100	100 (100)
Woodchuck	100	100 (100)
Dog	100	100 (100)
	100	100 (100)
Ornamental	100	100 (100)
Dwarf	100	100 (100)
Cat	100	100 (100)
	100	100 (100)
Frog	100	100 (100)
Geometric	100	100 (100)
Geometric	100	100 (100)
Rat	100	100 (100)
	100	100 (100)

As in the skin (Daniels 1959) the generation of that highly reactive and the fragments in eye tissues calls for a protective mechanism. The AA has previously been designated the role as participant in an electron transport system (H 1962) but so far a metabolic reaction for which that excessive transport capacity would be necessary in the eye has not been found. It is tempting to surmise therefore that the reversible redox reaction between AA and dehydro-AA is involved in taking care of radiation induced free radicals in the eye media (cornea, lens, retina) in general.

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CLINICAL EXPERIENCE WITH CONTINUOUS WEAR HYDROPHILIC CONTACT LENSES IN APHAKIA

BY

■ BARNER K MARNER and J A FAHMY

Twenty two aphakic eyes in seven females and thirteen males were fitted with a new type of soft contact lens designed for continuous wear. Nine patients were operated on for unilateral traumatic cataract and eleven patients were operated on for senile cataract. Sixteen patients terminated the study. In all cases the visual acuity with lens was almost equal to the acuity obtained with spectacles. The lenses were well tolerated without any special wearing schedule. No serious complications occurred. However, about one third of the patients developed conjunctivitis during the investigation period of eleven months. Four patients showed mild limbal vascular reaction while only one patient had neovascularisation along the corneal scar caused by the original trauma. The new lens was found to be a good alternative to other contact lenses.

Keywords: contact lenses - cataract - aphakia - infections

With the introduction of hydrated polymethylmethacrylate in the production of soft contact lenses by Wichterle & Lim (1960) a new era in this field was established since then many materials with several improvements have been presented.

The latest development seems to be materials designed for extended "permanent" wear. The initial success of continuous wear depends almost entirely on the oxygen permeability of the material (Fatt & St. Helen 1971, Leibowitz et al 1973, Fatt & Morris 1977). Most of the lenses used in previous clinical trials (Shaw & Gasset 1973, Jackson & Aquavella 1976, Pierse & Kersley 1976, Gasset et al 1977, Binder & Worthen 1977) were co-polymers of hydroxyethylmethacrylate (HEMA)

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or polymethylmethacrylate (PMMA) with polyvinylpyrrolidone (PVP) or ethyleneglycoldimethacrylate (EDMA) which provide low to moderate oxygen permeability (Ruben 1978).

The purpose of this investigation was to evaluate the clinical results of a new material (Duragel 75) with different physical and chemical properties. The material used is a complex multicomponent cross linked amido-amino copolymer with basically hydrophobic nature to which hydrophilic sites are grafted and it is unrelated to HEMA and derivative systems (Frankland & Highgate 1977, Ruben 1978). The data of the lens used in the present study are shown in Table I.

Material and Methods

The material comprises 20 patients (22 eyes) admitted to the Eye Department of Kommunehospitalet, Copenhagen from August 1977 to July 1978 for cataract surgery. Nine patients were operated on for unilateral traumatic cataract, and eleven patients were operated on for senile cataract. Two patients in the latter group were operated on both eyes.

During the trial period all patients who underwent surgery for traumatic cataract were offered to participate in the study, whereas patients in the senile group were selected in regard to cooperation and motivation. Patients with persistent corneal astigmatism exceeding 2 diopters were not included. The age and sex distribution appear from Table II.

The mean interval between operation and contact lens fitting was 19 weeks (range 8–26) in the traumatic group and 9 weeks (range 4–19) in the senile group.

Table I
Contact lens specifications

Name	Scanlens 24 H
Material	Amido-amino copolymer
Water content	76% (20°C)
Oxygen permeability	40.0 × 10 ⁻¹¹ units*
Optical transmission	97%
Refractive index	1.379
Centre thickness	0.4–0.5 mm
Base curves	7.9–8.1–8.5–8.5 mm
Diameter	13.5 mm

* units (cm² × ml O₂/sec × ml × mm Hg)

Table II
Age and sex distribution

Age	Traumatic		Semile		Total
	Male	Female	Male	Female	
<10	1	1			2
11-20	2				2
21-50	5				5
51-60			2	3	5
>60			3	3	6
Total	8	1	5	0	20

To avoid decentration fitting was performed steeper than recommended by the manufacturer (0.5 mm flatter than mean "K" reading) and was usually around the flattest "K" reading. After initial fitting the patients were examined with the hialamp after one h, 24 h, one week and monthly or more often when necessary. The patients were instructed not to remove the lens at any time and to contact the clinic in case of pain, discomfort or redness of the eye. The lenses were cleaned and soaked individually but mainly in the presence of discomfort and/or deposits.

Results

Twenty patients (22 eyes) were eligible according to the criteria described in methods. One patient was not included because of problems with the supply of an adequate lens. Nineteen patients were evaluable and with the exception of 3 patients were wearing the lenses throughout the period of investigation. These 3 patients were terminated before schedule because of recurrent deposits, fitting problems with resulting lens displacement and irritation of the eye. Thus 16 patients (18 eyes) were wearing appropriate lenses at the termination of the study.

Wearing period shown in Fig. 1 is the cumulative data for all lenses used in the different patients corrected for short interruptions; these interruptions represent 32 days in 6 patients and wearing period and observation period are then almost equal. Interruptions were caused by infectious conjunctivitis (30 days in 4 eyes) and by loss of lenses, cleaning and operation of the fellow eye (22 days in 3 eyes). The wearing period varied from one to eleven months. About 50% of the eyes had been wearing the lenses for more than six months at the termination of the investigation.

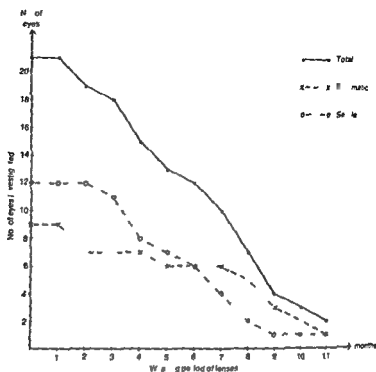


Fig 1

Wearing periods in months (the data are corrected for short interruptions specified in text)

Visual acuity was significantly the same when comparison was made between contact lenses and spectacles. Thus in 15 eyes the visual acuity was equal while in 6 eyes belonging to the traumatic group spectacles were slightly superior in 3 cases, and contact lenses slightly better in 3 other cases.

Table III
Causes of replaced lenses

Causes of replaced lenses	Traumatic	Senile	Total
Lost lenses	1	2	3
Broken lenses	1	1	2
Deposits	4	6	10
Fitting process	8	4	12
Total	14	13	27



Fig 2
Deposits on the surface of a lens

Replacement of lenses was done when necessary due to the causes listed in Table III. The relatively high number of lenses replaced in the fitting process was due to technical problems with the new type of lenses. Ten lenses were replaced due to deposits which was one of the most annoying problems. The table shows deposits to be responsible for replacement in about 2/3 of definite lenses and in 1/3 of all replacements. The deposits were round, glossy, whitish and prominent (Fig 3). The number ranged from one single large deposit to countless smaller spots. The durability of the lenses varied from 2 weeks to 10 months (average 5 months).

Complications from the wearing of the lenses were mainly of infectious and vascular nature. Infectious conjunctivitis was seen in 7 eyes (6 patients) all treated success-

fully with antibiotics and 4 eyes (3 patients) had concomitant interruption of wearing. Vascular reactions were found in five eyes only one in the form of neovascularisation but this could have been caused by the perforating trauma; the proliferation was seen in relation to the scar. Other vascular reactions (if any) appeared as moderate dilatation of the perilimbal vessels after 70–120 days of permanent wearing of the lens. No corneal or intraocular infections were seen in any of the eyes.

Discussion

The present results have demonstrated that the examined lens seems to be a good alternative to the other various types of contact lenses for the correction of unilateral aphakia.

From the practitioner's point of view the lens was relatively easy to fit, because of its large diameter and few curves (only 4 alternatives).

The lens was well tolerated without any special wearing schedule. Sixteen of the patients terminated the study which may be regarded as a high rate of wear success. The visual acuity with the lens was as good as the acuity obtained with spectacles which may indicate good optical properties.

Some shortcomings deserve attention. First the relatively short durability of the lenses which varied in average from 3 to 5 months. Second the relatively high frequency of deposits which were resistant to cleaning aids and were of great annoyance to the patients as well as to the practitioner. The relatively high number of control visits was considered another disadvantage.

There have been no serious complications in the present study such as corneal ulcers or intraocular infection as reported by some other authors (Herscovici 1977; Cooper & Constable 1977). However, nearly one third of the patients had infectious conjunctivitis which is in accordance with other reports dealing with continuous contact lens wear (Benson 1976; Gasset 1977). This fact may demonstrate the necessity of a careful instruction of patients to contact their ophthalmologist immediately in the case of pain, redness of the eye or haziness of sight.

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SOFT CONTACT LENSES WORN AT A SIMULATED ALTITUDE OF 18 000 FEET

BY

RUNE HAPNES

Five subjects wearing soft contact lenses were placed in a low pressure chamber where the atmospheric pressure was reduced to 50". Subjective and objective criteria were used to determine corneal distress. After four hours in the atmosphere all 10 eyes showed objective changes and four out of five subjects claimed subjective changes indicating corneal distress. None of the subjects showed subjective or objective signs of similar changes when the study was repeated at normal atmospheric pressure.

Key word: soft contact lenses - high altitude - corneal distress - corneal epithelial hypoxia

Like all other cells in the body, the corneal epithelial cells need oxygen to maintain normal function. In contrast to most other cells, they do not get their oxygen from the blood, but directly from the surrounding area through the pre-corneal tear film (Smelser & Ozanics 1952). In an open eye the partial oxygen tension in the tear film is roughly equal to the atmospheric oxygen tension, that is about 100 mmHg. When the eyelids are kept closed, the partial oxygen tension is reduced until it is in equilibrium with the oxygen tension in the palpebral capillaries - about 50 mmHg (Fatt & Bieber 1968). This is more than adequate to maintain normal corneal function.

When a contact lens is placed on the cornea, the pre-corneal oxygen tension is reduced by an unknown quantity. If the lens is too big, too tightly fitted or made of oxygen permeable lenses too thick, symptoms of corneal hypoxia occur. It has been estimated that the corneal epithelium needs a minimal oxygen

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nition of 11 to 19 mmHg to function normally over a longer period of time (Polse Mandell 1970)

A fully oxygenated cornea has oxygen reserves for about two hours continued normal function if all further oxygen supplies are prohibited (Untacke et al 1971)

Most of the contact lenses in common use today are aimed at being worn only for limited period of time usually not exceeding normal waking hours. One such lens type is the HEMA lens.

Patients with these lenses can wear them throughout the day without experiencing any discomfort but most of them will rapidly develop symptoms of overwear (= corneal hypoxia) if they fall asleep with their lenses on. It follows that a pre lens oxygen tension of 150 mm is enough to supply adequate oxygen to the cornea but a pre lens oxygen tension of only 25 mm is *not* sufficient. It has also been reported that patients with lenses that are fitted and functioning well at sea level can have problems at high altitudes where the oxygen tension is lower (Clarke 1976). This article aims at studying the effects of high altitude on lens covered corneas.

Materials and Methods

The study was performed in the low pressure chamber of the Norwegian Institute of Flight Medicine. In this chamber it is possible to control the atmospheric pressure and thereby the partial oxygen tension, the temperature, the air circulation and ventilation, light conditions and to some extent also relative humidity. Five people wearing well fitted soft contact lenses (HEMA lenses) on both eyes were selected for this experiment. They were aged from 20 to 40 years, four females and one male. They all had uncomplicated myopias ranging from -1.5 to -7 with corrected vision ranging from 6/5 to 6/8.5.

In the chamber the following parameters of corneal distress were recorded:

- 1. Subjective changes (fogging of vision and discomfort)
- 2. Changes observed with slit lamp (sclerotic scatter, ciliary injection, debris, corneal thickness changes)
- 3. Changes in visual acuity (with standard contrast and low contrast)

The five test persons and the examiner were fitted with tight fitting oxygen masks maintaining an oxygen supply equal to 150 mmHg.

When the above criteria of the cornea had been checked at normal atmospheric pressure, the pressure inside the tank was fairly rapidly reduced by half (simulating an altitude of 18 000 feet). The partial oxygen tension was just under 80 mmHg. The temperature, the humidity and the light conditions were kept constant during

Table I
Physical conditions in the chamber

Temperature	21 C
Relative humidity	41-45%
Chart illumination	5.5 lux
Draught	Negligible
Atmospheric pressure	1 atm ρ /1 851 mm
Atmospheric pressure control	1 atm ρ /1 256 mm

the experiment. The air circulation varied slightly but was kept well below the normally termed draught conditions. The contact lenses were kept on at all times during the experiment, also during the recovery period after descent to normobaric conditions. Slit lamp examination was performed with a Haag-Schmidt lamp fitted with a Maurice Giardini pachometer giving an accurate measurement ± 0.015 mm (3%). The cornea was examined first with a fine slit, then with sclerally scattered light; the limbus area was examined for injection, and the tear meniscus was examined for debris. Visual acuity was first tested with a standard Monover chart and then with a low contrast visual chart made of ordinary Snellen letters reflecting 80% of on falling light on a black background, reflecting 88% of on falling light.

Results

During the first two hours of the experiment no subjective or objective changes were observed. After about two hours three of the test persons developed debris in the tearfilm in both eyes, most easily observed in the tear meniscus at the upper margin of the lower lid. One of the test persons started to complain of slight corneal irritation at about this time. After three hours debris was observed in all ten eyes. After three hours three of the five persons experienced irritation in both eyes; after four hours one of the test persons was so uncomfortable that we had to interrupt the experiment. She had then rather pronounced conjunctival injection in both eyes. There was photophobia and abundant lacrimation.

No changes in the corneal thickness were registered during the experiment.

The visual acuity was reduced by one to three lines on the Monover chart in five out of ten eyes. It was unchanged in four eyes. The visual acuity on the special low contrast chart was reduced by one line or more in four eyes and was unchanged in six eyes. (One of the test persons who had unchanged visual acuity on the Monover chart had rather pronounced reduction of visual acuity on the low contrast chart.)

Table II
Subjective changes

		1 hour	2 hours	3 hours	4 hours
1 atm	Fogging of vision	0	2 eyes	6 eyes	8 eyes
	Discomfort	0	0	6 eyes	6 eyes
4 atm	Fogging of vision	0	0	0	0
	Discomfort	0	0	0	0

■ subjective changes were recorded continuously and in addition asked about specifically each hourly examination

The examiner who did not need optical correction and did not wear contact lenses experienced no subjective symptoms during the experiment

When the subjects were tested at normal atmospheric pressure but otherwise under the same conditions as in the first experiment no subjective or objective changes of significance were noted

Discussion

Tables II, III, and IV indicate all that subjects wearing this type of contact lenses at altitude of 18 000 feet may not tolerate their lenses as well as they do at sea level. Probably the main cause for this is corneal epithelial hypoxia. In this experiment no

Table III
Changes observed with the slit lamp

The slit lamp examination was made without removing the lenses thus making staining techniques inapplicable. For the same reason small degrees of sclerotic scatter were difficult to discern. Corneal thickness changes differing less than 0.03 mm from the start point value were recorded as 0.

		1 hour	2 hours	3 hours	4 hours
1 atm	Sclerotic scatter	0	0	0	0
	Ciliary injection	0	2 eyes	2 eyes	6 eyes
	Debris	0	6 eyes	10 eyes	10 eyes
	Corneal thickness	0	0	0	0
4 atm	Sclerotic scatter	0	0	0	0
	Ciliary injection	0	0	0	0
	Debris	0	0	0	0
	Corneal thickness	0	0	0	0

Table II

Changes in visual acuity

The visual acuity was measured on a standard Monovision chart and also on a special low contrast chart using Snellen letters. Visual reduction was only recorded when amounted to one full line or more.

		1 hour	2 hours	3 hours	4 hours
1 atm	Standard contrast	0	2 eyes	5 eyes	6 eyes
	Low contrast	0	2 eyes	4 eyes	4 eyes
1 atm	Standard contrast	0	0	0	0
	Low contrast	0	0	0	0

changes in corneal thickness were registered. Corneal thickness changes sufficient to cause significant change in the pachometry measurement would depend on stromal oedema. Our experiment was interrupted before there was time for stromal oedema to evolve. The symptoms and signs developed by all subjects indicate a corneal epithelial lesion but on slit lamp examination no definite oedema of the epithelium could be observed. This may partly be due to the obscuring effect of the contact lenses. The author believes that without laser sclerotic scatter techniques and staining techniques could show epithelial changes in keeping with the discomfort and visual reduction experienced by the patients in this study. This belief is supported by the increased amount of debris on the film which must originate from damaged epithelial cells.

The contact lenses in common use today decrease the oxygen available to the cornea by a considerable extent. This may cause practical problems for contact wearers travelling at high altitudes.

Acknowledgment

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THE SURFACE COAT ON HUMAN CORNEAL ENDOTHELIUM

BY

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Scanning electron microscopy of human cadaver corneas revealed a selective binding of ruthenium red-osmium tetroxide to some substance coating the posterior endothelial surface. A coating material was not found on endothelial cells on denuded areas of the membrane of Descemet or on the anterior surface of endothelial cells. Partial digestion of the coating material by urokinase and trypsin suggests the presence of at least three different structural or chemical elements.

Key words: cell coat - endothelium - human cornea - morphology - ruthenium red - scanning electron microscopy - trypsin - urokinase

A mixture of ruthenium red and osmium tetroxide precipitates some substance on the posterior surface of normal human corneas (Schmid & Sperling 1976; Jacobsen & Sperling 1978). A similar substance has been found between the trabecula in baboon, rabbit and human eyes (Segawa 1975; Harnish 1976; Green et al. 1977).

Ruthenium red-osmium tetroxide reacts with a number of negatively charged large polymers and with some lipids (Lust 1964, 1966, 1971a, b). The complex polymer-ruthenium red and osmium tetroxide is insoluble in ethanol and ethylenoxide. It can be studied by scanning and transmission electron microscopy.

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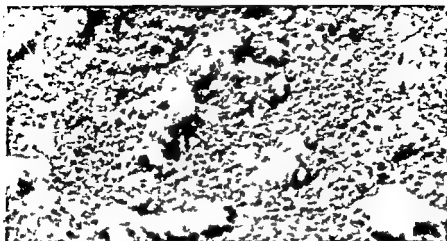


Fig. 1

The posterior surface of corneal endothelial cells. Fixed 14 h post mortem
Magnification 760 \times

Material and Methods

Whole human eyes were enucleated from cadavers stored at $1-20^{\circ}\text{C}$ for 8–10 h and later at 4°C . Seven eyes were obtained 8–24 h post mortem and two eyes 64 and 71 h post mortem. They were enucleated when no history of eye disease appeared from the case record and inspection revealed a normal cornea and a normal anterior chamber. Corneas were excised with a 1.5 mm rim of sclera. Three corneas obtained 64, 71, and 18 h post mortem were fixed immediately after excision. Three corneas were cut in thirds. Each third was incubated in urokinase (Leo 2000 IU/ml) in trypsin (Gibco 2.5 mg/ml) or in the basic medium without enzyme at 25°C for 15, 30, or 60 min. The enzymes were dissolved in a balanced salt solution (Ackroyd 1964). In three corneas the endothelium was partially peeled off the membrane of Descemet with a fine forceps under a dissecting microscope. Corneas were fixed in equal parts of ruthenium red (15 ppm in distilled water), 5% osmium tetroxide, and 0.2% cacodylate buffer (pH 7.3) for $1\frac{1}{2}$ h at 25°C . After fixation the tissue was transferred in cacodylate buffer, dehydrated in graded ethanol, critical point dried in carbon dioxide, vacuum coated with gold, and examined in a Cambridge Stereoscan 30 scanning electron microscope.

Results

In the corneas fixed immediately after excision, the posterior corneal surface of intact cells was covered by a coherent layer. The gross appearance of this layer is shown in Fig. 1. The resolution power of the microscope does not allow definite conclusions regarding the structure of the coating material, but high magnifications



Fig 2

Remnants of autolyzed endothelial cells on the membrane of Descemet's endothelium. Fixed 71 h post mortem. Magnification 3000x



Fig 3

The endothelium was partially removed from the membrane of Descemet's endothelium. The posterior surface of endothelial cells is visible. Middle: The area of endothelial removal on the everted edge of the coherent cell sheet. Right: The membrane of Descemet's endothelium. Fixed 12 h post mortem. Magnification 3000x



Fig 4

* anterior surface of a coherent sheet of endothelial cell after mechanical removal 16h post mortem Magnification 741x

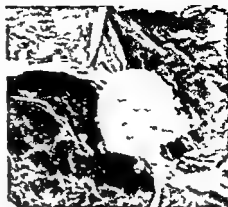


Fig 5

Larger magnification of a detail in Fig 4 Magnification 9600x

the impression that the layer was composed of spherical particles with diameters of 100–200 nm aggregated without preferred orientation (Jacobsen & Sjöling 1978)

On the two corneas obtained 64 and 71 h post mortem intact endothelial cells and partially autolysed cells were found. In some areas the slightly ruffled surface of the naked membrane of Descemet was visible. In other areas cellular debris and remnants of intercellular borders were found. No covering layer was found on fully or partially denuded areas of the membrane of Descemet or on autolysed cells (Fig 2). Nor was this material found on the anterior surface of the endothelium normally adhering to the membrane of Descemet (Figs 3–4–5).

On the anterior surface of the inverted cell sheet and on the membrane of Descemet the intercellular borders were clearly outlined (Figs 3–4–5). In some areas protrusions of the intercellular borders with diameters 2–6 μm and finely granular surfaces appeared (Figs 4–5).

On the corneas treated by urokinase for 60 min three different structural components could be discerned in the surface material. Spherical particles as those seen on the untreated surface were in some places overlying whirled branching fibers with diameters 200–250 nm (Fig 6). Adjacent to the cell surface branching straight fibers with diameters 100–150 nm were interwoven in a tight meshwork (Fig 7). In the corneas treated by trypsin for 30 and 60 min whirled fibers and altered remnants of coating material were found. In the controls incubated in the HEPES solution for 60 min some of the spherical particles were missing and the whirled pattern could be discerned.

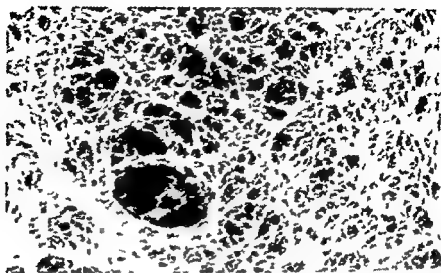


Fig 6

Whirled fibers L-rosinase 15 min Magnification 1000 x

Comments

A coating material was only found on the surface of intact endothelial cells in the anterior chamber. The lack of this material in other locations rules out the possibility that it is an artefact produced by the ruthenium red-osmium tetroxide alone. Our present concept of the structure of the ruthenium red-osmium tetroxide fixed surface coat is illustrated in Fig 8.



Fig 7

Stellate formations of straight fibers adjacent to the posterior cell surface L-rosinase 15 min Magnification 5000 x

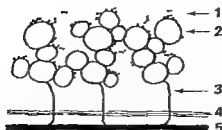


Fig 8

istic drawing of our present concept of the structure of the coating material after fixation in ruthenium red-osmium tetroxide 1 Spherical particles aggregated without red orientation seen in Fig 1 2 Fibers in loose whirls seen in Fig 6 3 Centers of the configurations of straight fibers seen in Fig 7 4 Straight fibers 5 Outer limiting cell membrane (Pnix Pro Faborg)

A delicate network of filaments between trabeculae in rabbit and baboon eyes was found in TEM pictures by Grierson et al (1977) when lower concentrations of ruthenium red were used in the fixation. Fibers were not observed by Schröder & Jørgensen (1977) in TEM preparations of the coating material.

The regular polygonal outline of the cell borders facing the membrane of Descemet was found in the present study while a jagged outline of cellular membranes was found close to the membrane of Descemet in rabbits by Hirsch et al (1977) when a fracture technique was applied. A jagged arrangement of the anterior cellular borders was also found in some human corneas after staining by ruthenium red trypan blue (Sperling 1977). These observations suggest that methodical or individual differences rather than species differences account for the discrepancies between the observed configurations of the basal cell borders in man and rabbits.

It is unlikely that the structure illustrated in Fig 8 represents the *in vivo* configuration of the coating material but this study indicates that at least three different structural or chemical components are present on the surface of intact endothelial cells.

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ENDOTHELIAL MORPHOLOGY RELATED TO DISEASE ACTIVITY IN HUMAN CORNEAS

BY

THOMAS OLSEN and STEFFEN SPERLING

Twenty normal and four groups of pathological corneas with the diagnoses aphakia, macula after herpetic keratitis, Fuchs' endothelial dystrophy and graft rejection were stained with trypan blue and alizarin red. The morphology of the endothelium was described in terms of cell density, coefficient of variation for cell area, percentage of hexagonal cells, percentage of joint meetings of more than three cells, nuclei per cell and nuclear shape.

The groups of aphakia, keratitis, Fuchs' dystrophy and graft rejection were considered to represent increasing degrees of endothelial disease activity at the time of evaluation. The only parameter showing consistent variation with disease activity was the percentage of joint meetings of more than three cells.

Key word: alizarin red - endothelium, cornea, morphology, trypan blue.

In normal human corneas a coherent monolayer of endothelial cells covers the anterior surface of the membrane of Descemet. Each cell is surrounded by four to five cells. The borders between neighbouring cells meet at angles approximating 120°. Pointed angles and joint meetings of more than three cells are rare. Each cell contains one globular or slightly oval nucleus (Sperling 1978a). When a single endothelial cell is damaged it is sloughed off the membrane of Descemet by neighbouring cells who in turn spread and cover the defect. The neighbouring cells radiate towards the center of the dead cell and form a joint meeting. Later the cells slide and reform a normal pattern. During reformation of the cellular pattern the cells occur in constellations characterized by converging

pointed angles and joint meetings of four and five cells (Oh & Evans 1961; Sævi 1976; Olson et al 1978; Sperling 1978; Olsen 1979).

After endothelial trauma or disease the numerical cell density decreases, cells become binucleate and multinucleate. Large variations in cellular size and shape appear (Nagano 1914; Stocker 1953, 1971; Binder & Binder-Chi et al 1960, 1962; Shierhölter & Honegger 1975). The percentage of hex cells has been shown to decrease after cataract extraction and with decreasing density in normal eyes (Olsen 1979a).

This study was undertaken in order to investigate whether deviations from normal endothelial pattern could be related to disease activity. For the purpose of this study four groups of corneas were selected. These groups were representative of different disease activities at the time of evaluation.

Material and Methods

Twenty normal and five aphakic eyes were enucleated from cadavers stored at 15 °C for 6–10 h later at 10 °C. Eyes were regarded normal when no history of eye disease was indicated in the case record and when the cornea and the anterior chamber appeared normal by simple inspection. Mean patient age 56.7 years (range 40–83). The aphakic eyes had been subjected to operation 4–9 years previously. Patient age ranged 61–74 years. Whole corneas were excised with a scleral rim. Endothelial cell borders and nuclei were stained with alizarin red and trypan blue (Sperling 1977). Six mm central buttons were cut from the end or side after staining. Eleven corneal buttons were obtained from patients undergoing penetrating keratoplasty. The diagnoses were: Macula after herpes keratitis (5), Fuchs endothelial dystrophy (3) and graft rejection (3). Patient age range in the three groups were 57–74 and 36–71 years respectively. The buttons were stained by alizarin red and trypan blue immediately after removal.

Buttons were bisected before wet mount on microscope slides with the endothelium to the cover glass. Photomicrographs were obtained on Kodak Tri-X film (74 × 36 mm) at magnification 40 × 8.5 (secondary enlargement to 12 × 10.8 cm). On central buttons from normal corneas the microscope was successively centered in predetermined points as described by Sperling (1978a) and six or more photographs were obtained of areas in which at least three quarters of a full field could be brought into focus. From pathological buttons four or more photographs were obtained from flat areas with cell cover.

For evaluation of cell morphology four photographs from each cornea were selected on the basis of the largest photographic clarity by a technician unaware of the purpose of the study. On each photograph a frame of 2 × 106 mm corresponding to a corneal area of 1 mm² was drawn in the area of the largest photographic clarity.

The following morphological characteristics were recorded: 1) Endothelial cell density estimated according to Sperling & Gundersen (1978). 2) Cell size. Cells from all patient corneas and from twelve normal corneas chosen at random were grouped according to point counting as described by Elias et al (1971). A point distance of 6.19 mm corresponding to 80 µm on cornea was chosen because this turned out to be the minimum necessary size. 3) Number of neighbours per cell. 4) Number of cells in joint meetings. 5) Number of nuclei per cell. 6) The ratio between the largest and the smallest nuclear diameter.

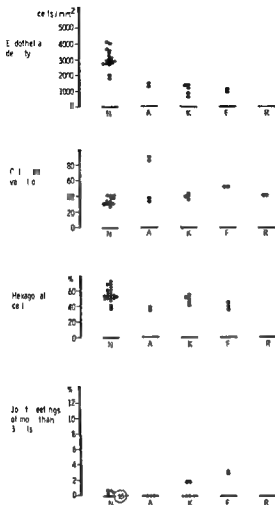


Fig 1

morphological characteristics of normal and pathological endothelial cells N = normal A = aphakia K = macula after herpetic keratitis F = Fuchs dystrophy R = graft rejection

$$\text{area variation} = \frac{S}{\bar{x}} \frac{100}{\bar{x}}$$

Results

In either pathological group the mean cell density was lower than in the normal group. In one normal cornea from a male patient of 50 years a cell density of 5634 cells/mm² was found. This finding is so unusual that this cornea was excluded from estimates of normal values. Mean numbers of cells per mm² (\pm SD) were: Normal = 19) 3101 \pm 732 aphakic 1254 \pm 363 keratitis 1080 \pm 303 Fuchs 1334 \pm

602 rejection 891 ± 716 cells/mm² (Groups N, A, K, F and R in Fig 1) but not correlated to cell density in the normal group ($r = -0.31$ $2P = 0.1$ $n = 19$)

In 12 normal and in either pathological group the cell area variation increased with increasing mean cell size. When the relative cell area variation (coefficient of variation $\frac{SD}{\bar{x}}$) was related to mean cell size this was not the case. For normal corneas $r = 0.21$ ($2P = 0.32$ $n = 12$). Coefficients of variation within the normal range were found in the keratitis group (K). Normal and high values were found in the dystrophy group (A) whereas slightly elevated values were found in Fuchs dystrophy (F) and in the rejected grafts (R).

The number of neighbours per cell was counted. Mean percentages of hexagonal cells were 58 ± 9 ($n = 19$), 43 ± 16 , 48 ± 5 , 39 ± 5 and 30 ± 9 per cent in groups A, K, F and R respectively (Fig 1). For either group a direct relationship existed between endothelial cell density and the percentage of hexagonal cells (Fig 3). In normal corneas $r = 0.53$ ($2P < 0.05$ $n = 19$).

The number of cells in joint meetings was counted. Joint meetings of four cells were found in five out of twenty normal corneas, in one out of five in the dystrophy group, in three out of five in the keratitis group and in all corneas in the dystrophy and rejection groups. In one cornea from the dystrophy group and in one from rejection group joint meetings of five cells were found. No joint meetings of six

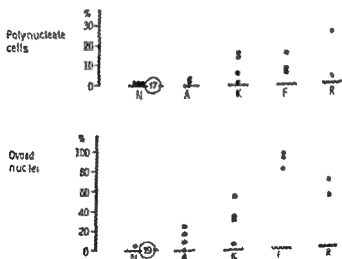


Fig 2

Nuclear characteristics of normal and pathological endothelial cells. N = normal, A = dystrophy, K = keratitis, F = Fuchs endothelial dystrophy, R = rejection. Ovoid nuclei signifies nuclei in which the ratio between the largest and the smallest diameter was $> 1.5:1$.

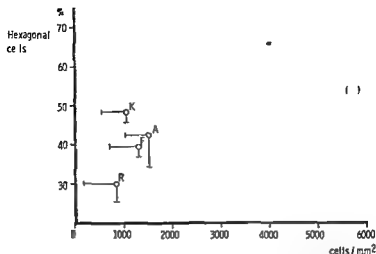


Fig 3

percentage of hexagonal cells related to cell density. Normal corneas are indicated by closed circles. For pathological corneas mean values are indicated by open circles. Standard deviations are indicated by bars. A = aphakia, K = macula after herpetic keratitis, F = Fuchs endothelial dystrophy, R = graft rejection. For normal corneas $r = 0.53$ ($2P < 0.05$, $n = 19$).

in five cells were found. Borders between cells in joint meetings of four cells met angles approximating 90° and these cells were surrounded by cells without converging pointed angles.

Nuclei per cell were counted and the nuclear shapes were recorded. Multinucleated cells and cells with nuclear diameter ratios > 1.5 were rare in the normal corneas but frequent in either pathological group (Fig 2).

Comments

Pathological corneas with differing disease activity were chosen for the present study. Aphakic eyes had been subjected to operation 4–9 years previously. No indication of corneal disease was found at the time of evaluation. In the corneas with macula after herpetic keratitis signs of acute inflammation had been absent for months. Fuchs dystrophy was chosen as an example of low grade current endothelial damage at the time of evaluation and graft rejection as an example of high grade current damage to the endothelium (Olsen 1979).

In order to indicate disease activity a morphological characteristic should vary gradually through the groups. Aphakia, keratitis, dystrophy and rejection. This was

not the case for cell density cell area variation percentage of hexagonal cells per cell or nuclear shape. The percentages of joint meetings of more than three cells increased with increasing disease activity. The highest values occurred in a group of rejected grafts and the lowest in corneas from normal and aphakic eyes. This suggests that the frequency of joint meetings of more than three cells may be regarded as an indicator of disease activity.

After injury of a single endothelial cell neighbouring cells radiate towards the centre of the damaged cell. The constellation of 5-8 neighbouring cells in a joint meeting (Figs 4b-4c) have been termed a *rosette* (Oh & Evans 1964). Deformation of rosettes the cells slide and occur in the constellations shown in Figs 4d-h. These constellations have been termed *reformation figures* (Sperling 1971). The patterns Fig 4d-h are characterized by cells with converging pointed angles and joint meetings of more than three cells.

The cells in joint meetings of four found in the present study did not appear to be reformation figure constellations. Borders between cells met at angles approximating 90° and they were surrounded by cells without converging pointed angles.

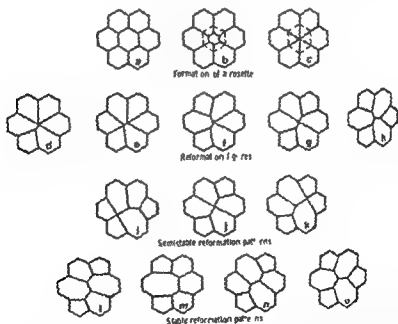


Fig. 4

a-c Formation of a *rosette* a joint meeting of six originally hexagonal cells. d-h *Reformation figures* formed by retraction of one, two or three cells (divided) from the rosette. i-k *Semistable reformation patterns* characterized by joint meetings of four cells and angles. l-o *Stable cell patterns* characterized by joint meetings of three cells and angles.

his observation suggests that rosettes are not necessarily reformed to joint settings of three cells within hours as reported by Sherrard (1976) and Sperling (1978). Intermediate semistable constellations of four cells without pointed angles in principle in Fig. 4 i-k may stay for extended periods of time before formation of stable cellular patterns (Fig. 4 l-o). In the present study it was found that the frequency of semistable cell constellations was correlated to disease activity.

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ON THE SIGNIFICANCE OF A LOW ENDOTHELIAL CELL DENSITY IN FUCHS' ENDOTHELIAL DYSTROPHY A SPECULAR MICROSCOPIC STUDY

BY

THOMAS OLSEN

In order to verify whether a low endothelial cell density is a primary event in Fuchs' endothelial dystrophy, a specular microscopic examination was attempted in 20 patients with Fuchs' dystrophy. Most of these patients had previously undergone cataract extraction. In five patients only was it possible in places to observe endothelial cellular outline to such an extent that the cell density could be estimated. The resulting estimates ranged from 115 to 2380 cells/mm² and were not as a group found to be lower than in a control group of aphakic subjects. This finding is evidence against the view that Fuchs' endothelial dystrophy is a low cell density syndrome.

Key words: cornea - endothelium - Fuchs' endothelial dystrophy - specular microscopy

Fuchs' endothelial dystrophy is a clinical diagnosis usually applied to the combination of corneal oedema and characteristic abnormalities in the posterior limiting layers of cornea as seen with the biomicroscope and called "guttae" after Vogt (10). In the past numerous attempts have been made to describe the pathological histological features of this disease, the site of which is generally considered to be the endothelium (Irvine 1956; Chis et al 1958; Stocker 1971; Iwamoto & de Voe 1971; Sella 1971; Hogan 1974). The finding of a low endothelial cell density has led many investigators to consider the compromised barrier function of the endothelium to be due to a low and inadequate number of cells. Such a hypothesis would

fit nicely with the association of Fuchs' disease with age and cataract extraction, of which factors are known to decrease the endothelial cell density.

Most studies on the endothelium in Fuchs' dystrophy have largely been on specimens removed at keratoplasty. That is, at the end stage of the disease it therefore be questioned whether the low cell density is a primary or a secondary finding in this disease. With the introduction of the specular microscope (A - 1968, Laing et al 1975, Bourne & Kaufman 1976, Sturrock & Sheppard 1979, Olsen 1979) it is possible to examine, photograph and quantitate the morphology of the endothelium *in vivo*. Therefore it is now theoretically possible to detect the earliest changes in the endothelium associated with Fuchs' disease.

The purpose of the present study was to present data on endothelial morphology in early stages of Fuchs' endothelial dystrophy as it is seen with a specular microscope. The aim of the study has especially been concerned with endothelial cell density in this disease.



Fig 1

The endothelial reflex in two patients with Fuchs' endothelial dystrophy. Top: 75 years of age, cataract extraction 2 years previously with 1 year history of corneal oedema. Central corneal thickness 0.60 mm. Estimated cell density 15 cells/mm². Bottom: woman 78 years of age, cataract extracted 15 years previously with 2 years of corneal oedema. Central corneal thickness 0.85 mm. Estimated cell density 11 cells/mm². Arrows point to the smallest defects in the reflex which showed a tendency to be linked to cell joints. Bar indicates 100 µm.

Subjects and Methods

total of 20 patients with Fuchs endothelial dystrophy were examined with the non-contactular microscope (Olsen 1979). They all fulfilled the following criteria (Waring et al 1979): 1) corneal oedema with bullae and 2) guttae present in the endothelium as seen with a slit lamp.

In the great majority of these patients endothelial cellular details were not detectable either due to extensive opacification of the cornea or absent reflex from the endothelium. In the remainder (3 patients) it was possible in places to obtain a number of photographs of discrete areas with visible cells. One of these patients, a 56-year-old male, had no other history of eye disease whereas the remaining four patients (age range 67-81 years) had had cataract surgery. Of these, one had formed one and a half, six and thirteen years prior to onset of the disease. In respect thereof, there was evidence of dystrophic changes present in the endothelium in all patients prior to the cataract surgery. The duration of symptoms prior to specular microscopic examination was between one half to four years.

Photographs were taken as centrally as possible (always more than about 3 mm from the limbus) along a horizontal line through the center of the cornea. This was done to ensure a constant distance from surgical wounds at the upper limbus. Counting of the cells was done as described earlier (Olsen 1979) by drawing a number of frames on the photomicrographs and using an unbiased counting technique. Due to the discrete areas in which endothelial cells could be seen, smaller frames had to be used, however. The total area of endothelium thus studied varied from 0.031 to the normally used area of 0.034 mm².

The use of the smaller sample area would tend to increase the standard error of the estimate by approximately the square root of the ratio between the smaller and the normal sample area, i.e. about thirty percent. Assuming a normal standard error of 4% (Olsen 1979), this would increase to 5.2% using the smallest sample area, i.e. still a rather low figure. As a control group, eight aphakic patients who successively came to cataract surgery on the other side were photographed and had their central endothelial cell densities estimated. None of these patients showed cornea guttata or had abnormal corneal thickness. Mean age 63 years, range 36-78. Time range after operation was between one half to three years.

Results

The typical specular microscopic appearance of the endothelium in Fuchs dystrophy was that of numerous confluent reflex-free areas (Fig. 1). In the majority of the examined patients these changes were so extensive that they concealed cellular details completely. In five patients it was possible to detect cellular details in areas of the endothelium large enough for estimates of the cell density to be drawn. These areas were detected by carefully scanning the endothelium and photographs were usually obtained at the periphery of the more centrally located oedema. At such points the reflex-free areas could be brought to vary in size by slightly adjusting the focusing depth or the angle of incident slit illumination, illustrating that they really were excrescences on Descemet's membrane and not cell-free or pigmented areas. A characteristic feature of the smallest reflex-free areas was the tendency to be located close to points where more than two cells met (arrows in Fig. 1).

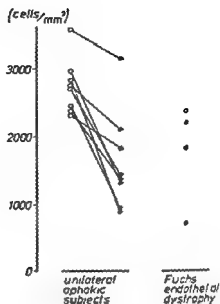


Fig 2

Estimated endothelial cell density in five patients with Fuchs endothelial dystrophy group of cataract extracted patients. Closed circles signify cataract extracted eyes. Open circles are from the same patient.

Estimated endothelial cell densities ranged from 715 to 2380 cells/mm² appear in Fig 2 together with estimates from the unilateral aphakic patients. It is seen that only in one instance did the cell counts from the group with Fuchs endothelial dystrophy fall below that of the aphakic group. The cell density of one patient without previous cataract extraction fell within the normal range of unoperated fellow eyes.

Discussion

The present study has shown that a decreased endothelial cell density is an obligatory finding in Fuchs endothelial dystrophy. This indicates the barrier function of the endothelium not to be simply a low cell density.

The five patients reported on in this paper were selected on the basis of the ability to observe the endothelium so that only patients without marked corneal disease and without completely deranged endothelium had their endothelial cell density estimated. These patients must be considered unrepresentative of the general group of patients with Fuchs endothelial dystrophy as compared to patients in which cellular details could not be observed.

It may be objected that the specular microscope only reveals areas with

rescences on Descemet's membrane so that the estimates of endothelial cell density are not representative of the whole endothelium. Flattening of cells over excrescences have also been described (Vogt 1930; Irvine 1956; Chi et al 1968; Kloucek 1967; Iwamoto & De Voe 1971; Hogan et al 1974). Therefore, theoretically, the increased permeability could still be explained as a low cell density localized at such points. In normal endothelia, if cells are damaged, neighbouring cells spread to cover the defect. Even after such immediate healing response, the endothelial cells have a capability of moving towards regions of lower cell density as has been shown after cataract surgery (Rao et al 1978). Therefore, if a decrease in endothelial cell density was the primary event in Fuchs' endothelial dystrophy, a certain smoothing out of differences in cell density would have occurred. The inability to demonstrate a low endothelial cell density in the present study therefore indicates that the disease is not initiated by a low number of cells *per se*. The cell densities reported in the present study are far above the lowest cell densities encountered in corneal grafts with normal hydration (Bourne & Kaufman 1976a). What really causes the disease remains unclear. Defects in the fibrinolytic system have been described (Bramsen & Stenbjerg 1979) but how the increase in fibrinolytic activity acts on the cornea is unknown. Evidence that the abnormal endothelial permeability is of intercellular origin may be found in the observed tendency for small reflex-free areas to be located close to cell meetings (Fig. 1). These small areas may be interpreted as localized subendothelial oedema pushing cell membranes posteriorly, whereby the light is reflected in other directions. Evidence that an active cell death is present has also been reported (Olsen & Sperling 1980). The low cell density seen in the end stage of the disease may therefore rather be the result and not the cause of the disease.

Acknowledgments

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TRIFLUORTHYMININE IN THE TREATMENT OF HERPES SIMPLEX CORNEAL ULCERS

A clinical evaluation

BY

PREBEN GILBERT and KRESTEN WORK

The treatment of herpetic corneal ulcerations with trifluorothymidine (F₃T) has been evaluated in a clinical trial. F₃T was found to be an effective antiviral agent for herpetic keratitis and a valuable alternative to idoxuridine (IDU) therapy. Clinically IDU-resistant herpetic ulcers responded favourably to treatment with F₃T.

Keywords: herpes simplex virus - keratitis - trifluorothymidine - idoxuridine

Treatment of herpetic corneal ulcers with idoxuridine (IDU) is unsatisfactory as a proportion fail to heal and some patients develop signs of toxicity or hypersensitivity to IDU. Unfortunately these are often the individuals most in need of antiviral therapy for herpes simplex keratitis. Trifluorothymidine (F₃T) has proved a significantly superior alternative to idoxuridine (Wellings et al. 1972) being uniformly effective in cases failing to heal with IDU therapy and in cases with a hypersensitivity reaction to IDU (McGill et al. 1974).

F₃T has a structure similar to that of IDU differing only in a side group. It is considerably less toxic to the cornea (Kaufmann & Herdelberger 1964; Holtmann & in 1977) and is also ten times more soluble. In tissue culture F₃T inhibits viral replication at the lowest concentration of any analogue yet tested (Umeda & Herdelberger 1969). In solution F₃T is rather unstable at neutral pH and storage for 4-5 days at 5°C reduces the concentration by approximately 50%.

The present investigation is a clinical evaluation of the effectiveness of F₃T in the treatment of herpes simplex corneal ulcers.

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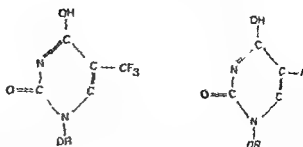


Fig 2

Structural formulae of trifluoromidine and idoxuridine (DR = deoxyribose)

Material and Methods

Consecutive typical cases of dendritic or amoeboid ulcerations of the cornea referred to the department over a 3 month period were included in the study. Twenty four patients had dendritic keratitis and five patients had amoeboid ulcerations. Nine patients received IDU treatment over a period of 4 weeks immediately before being taken into the F3T trial.

Treatment was carried out on an outpatient basis with 1% F3T. Crystalline F3T was dissolved in physiologic saline without preservative and sterilized by filtration. It was emphasized to the patient that one drop had to be instilled six times daily and that the bottle should be kept in the refrigerator. Due to the instability of the available F3T solution a fresh bottle was dispensed every four days throughout the period of treatment.

History and examination were recorded. The ulceration was drawn and photographed and the initial size estimated. Follow up examinations were performed as near to daily as possible for the first four days and thereafter on alternate days. The treatment was continued for a minimum period of eight days but was considered to have been completed when there was no visible ulceration or staining with either fluorescein or Bengal rose using the slitlamp.

Results

All dendritic ulcers healed within two weeks from the beginning of treatment with F3T.

Ten small dendritic ulcers (< 5 mm) healed in 2.8 days with an average of 1.5 days.

Fourteen large dendritic ulcers (≥ 5 mm) healed in 3.14 days with an average of 1.59 days.

the five amoeboid ulcerations healed in 7—18 days. Nine patients had what was considered to be IDU resistant ulcers and all healed with F3T after having been treated unsuccessfully with IDU for 1-3 weeks.

Discussion

In the present study we found F3T to be a valuable alternative to IDU in the treatment of herpetic keratitis. Most lesions healed within one week and we did not have any failures.

None of our patients with superficial epithelial lesions were seen to develop stromal keratitis. This might be due to the greater solubility of F3T as compared with IDU, allowing F3T to penetrate sufficiently into the cornea to inhibit virus replication deep in the stroma.

In this series we did not note any signs or symptoms that could be ascribed to toxicity of F3T. Nor did any of our patients develop any signs of allergy to the drug. This is very encouraging when dealing with a drug that seems to be considerably more active than IDU. F3T occasionally produces a contact dermatitis similar to that induced by IDU and in some cases signs of corneal epithelial toxicity will develop with the earliest sign being punctate erosions. If the treatment is continued, frank epithelial oedema without stromal swelling is produced. These signs quickly subside after F3T has been stopped (McGill et al. 1976). The absence of allergic and toxic effects in the present investigation is in accordance with other clinical and experimental studies. Thus Wellings et al. (1972) observed no side effects in 40 patients treated with F3T for a 2 week period. In eight patients with contact dermatitis due to IDU hypersensitivity, F3T was substituted and in no instance did a hypersensitivity reaction occur (McGill et al. 1974). In controlled animal experiments (Kaufmann & Heidelberger 1964) F3T did not produce toxic effects in the rabbit cornea when administered 2 hourly round the clock for two months. Kaufmann & Stein (1977) found no delaying effect of F3T on epithelial regeneration in the rabbit cornea.

Finally we noticed that generally the discomfort of the patients due to the herpetic affection disappeared after a few days of treatment and long before healing of the cornea was completed. Quite often the affected eyes were painless and the patients free of symptoms after one or two days of F3T treatment enabling them to resume their usual activities.

In conclusion we feel that F3T offers a valuable alternative to IDU therapy for herpetic ulcers.

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CLINICAL TRIAL OF THE TOPICAL USE OF DISODIUM CROMOGLYCATE IN VERNAL ALLERGIC AND CHRONIC CONJUNCTIVITIS

BY

MARKKU LEINO and ERKKI TUOVINEN

Thirty three patients were treated with disodium cromoglycate (DSCG). Of these 5 had vernal conjunctivitis, 18 had allergic conjunctivitis and 10 had mild chronic conjunctivitis in which the exact aetiology could not be determined. 70% of patients with DSCG as the only medication achieved at least some beneficial effect being almost exclusively in the groups of either vernal or allergic conjunctivitis. The basic trend seemed to indicate a reduction in severity during the treatment with DSCG in allergic diseases.

Key words: vernal keratoconjunctivitis - allergic conjunctivitis - chronic conjunctivitis - disodium cromoglycate

The therapeutic efficacy of disodium cromoglycate (DSCG) in bronchial asthma is generally accepted. The administration of DSCG has reduced the requirement for steroid therapy in many patients with chronic asthma. It is often very useful in exercise induced asthma, but it can be helpful in all types of asthma.

DSCG inhibits the release of chemical mediators in immediate IgE mediated hypersensitivity, particularly those involving mast cells. DSCG is thought to stabilize in some way the outer layers of mast cells (Mansell et al.). It is suggested that DSCG interferes with Ca transport across the mast cell membrane (Foreman et al.).

The study presents the results of a clinical trial with DSCG in patients with vernal allergic or mild chronic conjunctivitis.

Material and Methods

Thirty three outpatients referred to the Department of Ophthalmology at the University Hospital of Kuopio during May 1977 to July 1978 were treated with Lomudal® eye drops which were not yet registered for general use in Finland at the time of the trial.

The diagnoses of allergic conjunctivitis were based on a positive history and conjunctival scrapings for eosinophilic leucocytes. In the allergic group 8 patients had negative scrapings for eosinophilic leucocytes but the symptoms and history suggested an allergic etiology. In addition bacterial and chlamydia cultures were performed. Two patients who had positive chlamydia cultures (with negative scrapings for eosinophilic leucocytes) were excluded from the trial. One patient in the allergic group had a scanty conjunctival growth of *Staphylococcus aureus*. Besides the latter patient none of the patients in the trial had either positive bacterial or chlamydia cultures.

The five patients with vernal conjunctivitis had all previously received topical steroid therapy. Similarly, most of the 18 patients with allergic conjunctivitis and the 10 patients with mild chronic conjunctivitis had previously received symptomatic and anti-allergic topical and systemic medication. Observation time varied from less than a week to 30 weeks averaging a good seven weeks. In patients with an allergic conjunctivitis had a short observation time because of a prompt favourable response and due relief of symptoms with the recession of the allergic season. In one case of chronic conjunctivitis an apparently harmful effect led to the discontinuance of treatment only a few days after commencement.

The drops were instilled usually four times daily in both eyes although in patients with vernal conjunctivitis often 5-6 times a day. The patients were inquired for the following symptoms: itching, soreness, grittiness, discharge, photophobia and pain. Six patients also received topical steroids and/or other anti-allergic medication (Table I).

Results

The patients with either vernal or allergic conjunctivitis responded favourably to the treatment, most of whom became either symptomless or nearly symptomless during treatment. Two patients with mild allergic conjunctivitis received no apparent relief of symptoms. In the chronic conjunctivitis group the response was generally insignificant.

At the end of observation time the treatment was discontinued for most of the chronic conjunctivitis patients because of insufficient clinical response.

Table I
Control of eye disease with Lomudal*

	Fully controlled	Partly controlled	Useful response	Poor response	No response
<i>nal conj</i>					
DSCC alone	11	0	0	0	0
DSCC + other therapy	1	2	0	0	0
<i>ergic conj</i>					
DSCC alone	9	2	3	1	1
DSCC + other therapy	1	1	0	0	0
<i>r nte conj</i>					
DSCC alone	1	2	0	3	3
DSCC + other therapy	1	0	0	0	0

ully controlled = complete or nearly complete relief of symptoms Partly controlled = a distinct relief of symptoms but patient not completely satisfied Useful response = some relief of symptoms but patient not nearly satisfied Poor or no response = no distinct relief of symptoms

Table II
Severity of eye disease before treatment and at the end of observation time

Severity	Vernal conj		Allergic conj		Chronic conj		Total	
	Before	After	Before	After	Before	After	Before	After
Absent	0	2	0	11	0	3	0	10
Mild	0	3	7	~	5	6	12	16
Moderate	3	0	~	0	5	1	15	1
Moderate/Severe	1	0	2	0	0	0	3	0
Severe	1	0	2	0	0	0	3	0
Total	11	11	18	18	10	10	33	33

bsent = no symptoms Mild = discomfort and inconvenience just noticeable Moderate = discomfort and inconvenience most of the day but does not interfere with daily routine Moderate to severe = discomfort and inconvenience noticeable most of the day and interference with normal daily activity (e.g. work reading watching TV driving) Severe = total daily routine disrupted

continued for over half (10 out of 18) of the allergic conjunctivitis patients and continued for all of the vernal conjunctivitis patients.

In many cases the allergic season was over at the end of observation time and treatment was no longer required in the allergic conjunctivitis group of patients.

A useful response was observed in 21 patients in the allergic and conjunctivitis group but only in four patients in the chronic conjunctivitis group. A statistically significant ($P < 0.005$) difference exists between these two groups (contingency method).

Table II conveys a general trend towards improvement in the vernal and allergic conjunctivitis patient groups. The side effects which were encountered in the patients were mild foreign body or burning sensations on application of the medication which led to discontinuance of the medication in the other patient.

The results of this study conform with results obtained in other trials with sodium cromoglycate (Easty et al., Greenbaum et al., Kazdan et al., Tabbara et al.). The spontaneous remission of vernal disease was a surprise possibly related to the Northern location of the study where the trial was carried out.

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TRAUMATIC HYPHAEMA TREATED AMBULATORY AND WITHOUT ANTIFIBRINOLYTIC DRUGS

BY

A. K. SJØLIE and K. KAMP MORTENSEN

Forty-four patients referred consecutively over a period of one year for treatment of traumatic hyphaema have been treated ambulatory and with no drugs.

Four patients developed secondary haemorrhage; three regained normal visual acuity.

The frequency of secondary haemorrhage is not statistically different from that in two previously published patient groups treated with bed rest and with tranexamic acid, respectively.

Key words: traumatic hyphaema – secondary haemorrhage – antifibrinolytic treatment.

The treatment of traumatic hyphaema has been aimed in particular at the prevention of secondary haemorrhage.

We have previously (1978) reported the results in a group of patients treated ambulatory and with tranexamic acid as compared to a group of patients treated with bed rest without being able to demonstrate any statistically significant difference in the frequency of secondary haemorrhage.

Since then we have treated patients with traumatic hyphaema without rest in bed in principle as outpatients and without any antifibrinolytic drugs. The results of a one-year treatment period of a relatively small number of patients are presented, as these results have not previously been reported.

Material and Methods

Forty four patients were referred consecutively to the Eye Department Odense University Hospital during the period 1/1-31/12 1978 for traumatic hyphaema.

In cases of uncomplicated hyphaema the patients were followed, ophthalmological examination prescribed rest for one week as the only treatment consisted of no reading or greater physical exertion and one week sick leave work.

The patients were subjected to control examination at the Eye Department 5th and 12th days after the accident further they were given instructions in the department should a reduction in vision or pain in the eye occur. If patients below the age of 12 years were treated as ambulatory patients in ward. Eleven patients with increased intraocular pressure serious lesions of bleeding within the corpus vitreum or with lesions of the retina were also referred to the department and treated as ambulatory patients.

The age and sex distribution of the patients are shown in Table I.

Table II shows the distribution of the accompanying lesions as compared in a group of patients treated with tranexamic acid (reported in a previous investigation Mortensen & Sjolie 1978). The distribution of the accompanying lesions in the two groups is very similar suggesting comparable severe traumas.

The chi-square test with Yates' correction has been employed for the analysis and 95% confidence limits have been used.

Table I
Age and sex distribution of 44 patients with traumatic hyphaema

Age	Female	Male	Total
0-9	1	4	5
10-19	2	11	15
20-29	1		8
30-39	1	1	7
40-49	0	3	4
50-59	11	1	2
60-69	0	3	3
Total	5	31	44

Table II

Accompanying lesions in 44 patients treated without tranexamic acid and 64 patients treated with tranexamic acid suffering from traumatic hyphaema

Accompanying lesions	-tranexamic acid	+tranexamic acid
Cornea and conjunctiva	25	31
Pupil and iris	27	51
Increased IOP initially	4	7
Retina and vitreus	8	13
Traumatic cataract	0	1
No accompanying lesions	7	3

Results

Of the 44 patients developed secondary haemorrhage giving a frequency of 2 (2.3% 21.7%)

In these four patients with secondary haemorrhage the rebleeding took place in one on the first day in one on the second day and in two on the third day after the trauma

At the final examination 43 patients had a visual acuity of $\geq 6/9$ in the affected eye of these 3 had suffered from secondary haemorrhage

One patient had a visual acuity of only 0.5/36 five months after the trauma. A secondary haemorrhage developed on the third day after the injury and the case was further complicated by an increased intraocular pressure transient myochromatosis fibrinous iritis as well as corpus vitreum bleeding with permanent opacities in the corpus vitreum

Discussion

There is little agreement as to the prognostic importance with regard to vision of secondary haemorrhage following traumatic hyphaema. Neither is there agreement on the aspect of the frequency of occurrence

Some authors (Henry 1960 Edwards & Layden 1973) thus state that the reduction in visual acuity results from lesions accompanying the initial trauma itself while others (Gregersen 1962) report that the prognosis with regard to vision is considerably poorer following the occurrence of secondary haemorrhage. Accompanying lesions causing a reduction in vision are considerably more frequent in American than in Scandinavian studies. This would suggest a difference in the

severity of the trauma. Such a difference in the composition of the material may explain the non uniform evaluation of the importance of secondary haemorrhage.

Crouch & Frenkel (1976) carried out a double blind investigation of the frequency of secondary haemorrhage in 59 patients treated with either epsilon-aminocaproic acid or placebo. Rebleeding was found in 3% of the patients with aminocaproic acid while 33% of the placebo group suffered from rebleeding. A difference which was statistically significant.

Bramsen (1976) found in 72 patients treated with tranexamic acid and in only one patient (1.4%) with secondary bleeding. In a subsequent publication (1977) rebleeding was observed in 75 patients treated ambulatory and with tranexamic acid.

Mortensen & Sjolie (1978) found secondary haemorrhage in 5% of 55 severely treated patients and in 0% of 64 patients treated ambulatory with tranexamic acid. The occurrence of secondary haemorrhage in the present investigation was 9.1% and statistical analysis of the three above mentioned investigations demonstrated no significant difference in the frequency of rebleeding in traumas evaluated from the accompanying lesions were not particularly severe. This can be seen from Table II.

Rest in bed has previously been the preferred type of treatment in the traumatic hyphaema and it has been postulated rather than demonstrated that rest has a favourable effect on the frequency of secondary haemorrhage.

The results of this investigation show that the patients have been adequately treated as ambulatory patients. In our opinion there is insufficient evidence present to indicate whether or not antifibrinolytic agents should be employed prophylactically in the treatment of traumatic hyphaema.

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REFRACTIVE ERRORS AND OTHER OCULAR FINDINGS IN SCHOOL CHILDREN

BY

L. LAATIKAINEN and H. ERKKILÄ

A series consisting of 411 non-selected school children 7 to 15 years of age was examined. Decreased visual acuity (less than 0.8 in one or both eyes without correction) was found in 55 children (13.4%) the frequency increasing from 3.7% to 29.1% with age. The frequency of hyperopic eyes (+2.0 D or more) decreased from 19.1% to 3.6% and the frequency of myopic eyes (-0.5 D or more) increased from 1.9% to 21.8% with age. Altogether myopia, hyperopia or astigmatism (1 D or more) in one or both eyes was found in 88 children (22.6%).

Manifest strabismus was found in 19 children (4.6%) and heterophoria (8 prism dioptres or more for near or distance) in 28 cases (6.8%) increasing significantly with age from 1.2 to 13.6%. Amblyopia (visual acuity 0.6 or less) due to strabismus or refractive errors was found in 5 cases. Subnormal vision due to congenital nystagmus, unilateral macular scar, congenital rubella retinopathy and unilateral Coats' disease was diagnosed with one case of each. Signs of external inflammatory disease were seen in 13 cases.

In this series the annual need for ophthalmological consultation increased from 5% at the age of 7-8 to 30% of the children aged 14-15 if follow up examinations and visits for the prescribing of glasses were included.

Keywords: refraction - refractive errors - visual acuity - strabismus - child - prevalence

ording to the 1972 law on basic health services in Finland all school children it be given regular health examinations including screening of vision. No recent lemiological studies concerning the prevalence of refractive errors and other lar disorders have been carried out on Finnish school children and the annual d for ophthalmological examinations is not fully known.

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Comprehensive epidemiological ophthalmological studies of school children have been made in the USA (National Health Survey 1972) and in Germany (1978). In addition there are reports concerning the epidemiology of ocular conditions e.g. refractive errors (Goldschmidt 1968), visual acuity (Ham et al. 1978) and squint (Frandsen 1960, Rantanen & Tormala 1971) in different populations.

The purpose of the present study was to estimate the prevalence of the ocular disorders and to ascertain the annual need for ophthalmological examinations among school children.

Subjects and Methods

In order to obtain a representative sample of the average child population in southern Finland, the study was carried out on comprehensive school children in all the communities in the county of Uusimaa, excluding Helsinki and its surroundings. The number of children invited from each community was proportional to the community's percentage of all the comprehensive school children within the study area. One to three whole classes per community depending on the number of children desired, were selected at random. Classes I (7-8 years), III (9-10 years), V (11-12 years) and VIII (14-15 years) were invited, and were represented by an approximately equal number of pupils. Some communities refused to have their pupils to be examined, and this led to a slight inequality between the age groups.

The final sample included 23 classes. For the examination the children were transported in small groups from the school to the outpatient department of the Helsinki University Eye Hospital. Two children who were not at school were

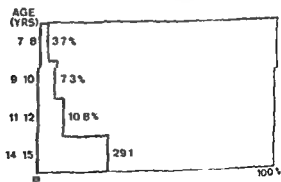


Fig. 1

Percentage of children with visual acuity of less than 0.8 in one or both eyes in the age groups.

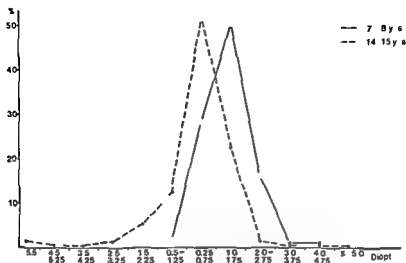


Fig 2

bution of refractive errors discovered by retinoscopy during cycloplegia and expressed in spherical equivalents in the youngest (7-8 years) and oldest (14-15 years) age group

the examination were excluded from the series. The final sample consisted of children representing the four age groups: 7-8 years 81, 9-10 years 109, 11-12 years 111 and 14-15 years 110 pupils.

411 children were given a thorough ophthalmological examination including testing of visual acuity for distance with the Snellen charts, streak retinoscopy both out and with cycloplegia (40-60 min after instillation of 1% cyclopentolate in each eye), testing of eye movements and cover test followed by prism cover if phoria or tropia was detected. Biomicroscopy of the anterior segment and direct and indirect ophthalmoscopy were performed in all cases.

Results

Visual acuity

Decreased visual acuity (less than 0.8 in one or both eyes without correction) was found in 55 of the 411 children (13.4%). The percentage of pupils with decreased vision increased from 3.7% in Class I (7-8 years) to 29.1% in Class VIII (14-15 years) as shown in Fig. 1. In 48 of the 55 cases the visual acuity could be corrected with glasses. Five eyes (1.2%) were amblyopic; in two cases this was due to strabismus, in two to anisometropia and in one to high astigmatism. In four of the amblyopic eyes visual acuity was between 0.2 and 0.6, thus deep amblyopia.

(visual acuity 0.1 or less) was found in one eye only. Other causes for decreased visual acuity were congenital nystagmus in one child and a macular chorioretinal scar in one child.

Refraction

A difference of one dioptre or more between the refractions measured with and without cycloplegia was found in 57 of the 411 subjects (13.9%). In the following measurements obtained during cycloplegia are used.

The distribution of the refractive errors (expressed in spherical equivalent) in the youngest (7-8 years) and the oldest (14-15 years) group are shown in Fig. 1. The corresponding curves of the other two age groups, if drawn, would be between these two curves. These curves reveal a general decrease in hyperopia and an increase in emmetropia or myopia with age. Thus the percentage of moderate to high hyperopia ($+2.0$ D or more) decreased from 19.1% in the youngest to 3.6% in the oldest age group (Table 1). In contrast, the percentages of myopic eyes (0.1% or more) increased from 1.9% in the youngest to 21.8% in the oldest group (Table 1), with a steep rise after the age of 11-12 years. The decrease in hyperopia and the increase in myopia with advancing age were both highly significant ($P < 0.0005$, $\chi^2 = 24.4$ and 74.594 respectively). Astigmatic errors of 1 D or more were found in 11 children (1.7%) and anisometropia of more than 1 D between the eyes was present in 11 cases (3.6%). The percentual share of the eyes with the various refractive errors in the whole series is shown in Fig. 3. Altogether myopia of -0.5 D or more or hyperopia of $+2.0$ D or more or astigmatism of 1 D or more in one or both eyes was found in 93 children (22.6%) and 58 (14.1%) of all the children needed glasses. Glasses were usually not prescribed in monocular myopia.

Table 1
Distribution of hyperopic ($\geq +2.0$ D) and myopic (≤ -0.5 D) eyes by age groups

Age group (years)	Total number of eyes	Hyperopic eyes		Myopic eyes	
		No.	%	No.	%
7-8	162	31	19.1	3	1.9
9-10	218	15	6.9	14	6.4
11-12	222	26	11.7	16	7.2
14-15	220	8	3.6	48	21.8
Total	822	80	9.7	81	9.9

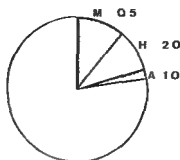


Fig 3

Proportion of eyes with myopia ($M \leq -0.5 D$), hyperopia ($H \leq +2.0 D$) and astigmatism ($A \leq 1.0 D$) in whole series

moderate hyperopia (cycloplegic refraction $+3.0 D$ or less) wearing of glasses was considered only if strabismus, amblyopia, anisometropia, astigmatism or subjective symptoms related to near work were found.

Strabismus

Manifest strabismus was found in 19 of the 411 children (4.6% Table II), esotropia in 12, exotropia in 7 children. The prevalence of manifest strabismus showed variation between the age groups and there was no significant correlation with increasing age ($\chi^2 = 4.8493$, $0.1 < P < 0.2$). Twelve of the children wore glasses, eight had been operated on and in three cases surgery was being considered because of a large angle of deviation.

Heterophoria of 8 prism dioptres or more for near or distance was found in 28 subjects (6.8% Table II), esophoria in 11 (2.2%) and exophoria in 19 (4.6%) cases.

Table II
Presence of strabismus by age groups

Age group (years)	Manifest strabismus		Heterophoria	
	No.	%	No.	%
7-8	2	2.5	1	1.2
9-10	3	2.8	4	3.7
11-12	9	8.1	8	7.2
14-15	5	4.5	15	13.6
Total	19	4.6	28	6.8

There was a gradual increase in the frequency of heterophoria from 12% youngest to 13.6% in the oldest age groups which was statistically highly significant ($\chi^2 = 13.761$ $P < 0.005$). Four of the 9 children with esophoria wore glasses because of hyperopia. The other 5 had no symptoms related to esophoria. The 19 children with exophoria had intermittent diplopia. In 8 children in those 3 wearing of glasses was considered also because of the refractive fusional reserves were not measured but the near point of convergence was pathological (more than 10 cm from the nose up) only in one child who however was symptom-free. One child had been operated on because of heterophoria. At present surgery was not considered in any of them.

Other findings

Signs of external eye disease were found in 13 children: chronic conjunctivitis in 4 and follicular conjunctival hyperplasia in 9 cases. Only one age group had symptoms related to these findings.

In one eye Coats's disease was diagnosed and one child had rubella retinopathy and iris hypoplasia. Twenty-two eyes (27%) showed diffuse coarse pigmentation of the macula or the whole fundus although visual acuity was normal. In pigmented changes in the retina were seen in 9 eyes: grouped pigmentation (pigment clumping) (2 eyes) or isolated pigment epithelial naevus (5 eyes). A choroidal naevus was found in 3 eyes. Characteristics of the optic disc are presented in another paper (Erkkila & Laatikainen 1979).

Findings in 18 children with a birth weight of 2500 g or less were examined separately. The only finding in which they differed significantly from the others was the prevalence of manifest strabismus which was found in 6 of the children or 33% ($\chi^2 = 35.196$ $P < 0.0005$).

Discussion

Refractive errors and strabismus are the main reasons for ophthalmological consultations at school age. During childhood there is a general shift in refraction towards decreased hyperopia or emmetropia and in some eyes this change may lead to the development of myopia (Sorsby et al. 1957). From the age of 11-15 years this shift was found to be about one dioptre (Fig. 1) at age of 11 the peak of the curve was between +1.0 and +1.75 D and at age of 14-15 years between +0.75 and -0.25 D.

The prevalence of myopia varies in different countries and populations. In 1880 Florschütz in Germany reported that 7% of the children were myopic whereas in the 10th class the share of myopes was 21%.

sponding age groups the frequencies of myopic eyes during the first decades of the century in Finland were 1.26% and 13.6% (Heinonen 1927-1934). In the present study the prevalence of myopia at the age of 7-8 years (1.9%) corresponded to that found by Heinonen (1927) but at the age of 14-15 years the proportion of myopic eyes was considerably greater (21.8%) in the present than in the earlier study (Heinonen 1934). This seems to indicate that the frequency of myopic refractive errors has increased during the last decades.

The prevalence of manifest strabismus in the whole series (4.6%) corresponded to that found earlier in a Finnish series of 7-8 year-old children (Rantanen & Tommila 1971). The frequency of heterophoria, exophoria, particularly increased with age. In most cases heterophoria was only of moderate severity and asymptomatic. The prevalence of amblyopia (1.2%) was slightly less than that reported by Rantanen & Tommila (1971) (1.9%) probably indicating the earlier diagnosis and treatment of refractive errors and treatment of strabismus.

In this series more than 20% of the children had refractive errors of significant degree in one or both eyes and 14% wore or needed glasses. Increase in myopia was the main cause for the considerable age-related increase in the demand for ophthalmological examinations. At the age of 7-8 years about 5% of the children had to have been sent for ophthalmological consultation, whereas the corresponding figure at the age of 14-15 years would have exceeded 30%, approximately the same as was also reported by Hamilton (1974) in USA. On the basis of the present study it was estimated that about 15% of all school children would annually be found to need an ophthalmological examination if the follow-up examinations and referrals for the prescription of glasses were included in the calculations. However, at one-fifth of them might not require any treatment.

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VISUAL SCREENING OF SCHOOL CHILDREN

BY

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A non selected group of 411 school children 7 to 15 years of age were screened by the school nurses. The screening program included (1) testing of visual acuity by the Snellen chart (with glasses if worn) (2) observing noticeable strabismus and (3) recording subjective ocular symptoms related to reading. Later the whole series underwent a thorough eye examination performed by the authors including a hyperopia test and the Titmus stereo test.

About 15% of the children failed to pass the screening in schools. 10.0% had visual acuity of less than 0.8 in one or both eyes with glasses, in another 1.0% obvious strabismus was observed and 4.1% complained of ocular symptoms. The same screening procedure at the final examination revealed another 2% of children with decreased vision, most of them slightly myopic.

By hyperopia test 57.0% of children with cycloplegic refraction of +2.0 D or more were discovered, more than half of them failed also at the present screening. 2.9% of those with mild hyperopia (<+2.0 D) showed positive hyperopia test. By the Titmus test all but one with manifest strabismus were discovered, whereas children with mild amblyopia passed. Defective stereopsis without other abnormalities was found in 11% of the children.

Keywords: screening—visual acuity—strabismus—stereopsis—child

Screening of vision is an integral part of the regular health examinations provided school children and carried out by the school nurses. In Finland visual screening is usually performed in the first, third, fifth and eighth classes. Children in other series are also examined if ocular symptoms appear.

The methods of screening and criteria for sending the children for further ophthalmological consultation are not uniform and their effectivity and practicability have not been assessed. In this report the results of screening by the methods

currently used are presented and compared with those obtained by elementary methods proposed.

Subjects and Methods

This study was a part of an epidemiological investigation where the prevalence of ocular disorders and the annual need for ophthalmological examinations of school children were examined (Laatikainen & Erkkila 1990). The study covered 12 school classes totalling 411 children 7 to 15 years of age described in detail in the previous paper.

Before the study the school nurses were asked to examine the children by testing the visual acuity of both eyes separately for distance using the Snellen chart with and without glasses, (2) observing noticeable strabismus and (3) recording subjective ocular complaints related to reading or other near work. The nurses filled a form for every child including the main criterion for further ophthalmological examination whenever present. The criteria given were (1) decreased visual acuity of less than 0.8 in one or both eyes (with glasses if worn), (2) noticeable strabismus and (3) ocular symptoms related to near work.

All 411 children were later examined by the authors at the outpatient department of Helsinki University Eye Hospital. After testing of visual acuity for distance without and with previous glasses, a hyperopia test and the Titmus stereopsis test were performed in all cases. The hyperopia test was regarded positive if after 5 min adaptation time the child was able to read correctly the Snellen figures corresponding to the visual acuity of 0.8 binocularly with +2.0 D lenses in the trial frame. Cycloplegic refractions and results of the other examinations have recently been presented in detail in a previous paper (Laatikainen & Erkkila 1990).

Results

Screening of vision using the present methods and criteria

Visual acuity of less than 0.8 in one or both eyes (with previous glasses if prescribed) was found in 41 children (10.0%) by the nurses (Table 1). At the ophthalmological examination this criterion was found to occur in 19 cases (4.6%). Nine of these children had passed the same screening in schools. Eight of these children were slightly myopic (-0.5 to -1.25 D) and one was highly hyperopic (+5.0 D) in both eyes. False positive result in the nurse's screening was found in one case only.

Strabismus was the main criterion for further ophthalmological examination in 10 cases (2.4%) and ocular complaints related to reading or other near work in 41 cases (10.0%).

Table I
Results of screening performed by school nurses

Criterion	Number of children	%
Visual acuity < 0.8 in one or both eyes with glasses	11	10.0
Strabismus	4	1.0
Ocular complaints related to near work	17	4.1
Total	69	15.1

Altogether the number of children who failed at the screenings was 15.17% of whole series but one fifth of them (18.6%) did not require any treatment.

Hyperopia test

In the whole series 34 children had hyperopia of +2.0 D or more (cycloplegic refraction) in one or both eyes. In 20 of them (37.0%) the hyperopia test was positive (binocular vision 0.8 or better with +2.0 D glasses) (Table II). Eleven of the 20 were also discovered by the screening methods currently used. All were symptom free and passed the screening. A positive result in the hyperopia test was also received in 8 children with hyperopia of less than +2.0 D (2.2%).

Table II
Relation between hyperopia (cycloplegic refraction of the more hyperopic eye), hyperopia test (binocular vision 0.8 or better with +2.0 D glasses) and present screening methods (visual acuity of less than 0.8 in one or both eyes, obvious strabismus or ocular complaints related to near work)

Refraction	Total number of children	Number of children with positive hyperopia test		
		Total	Failed at present screening	Passed present screening
+2.0 - +2.75 D	33	6	2	4
≥ +3.0 D	19	14	9	5
Total	44	20	11	9

Titmus stereo test

Stereoscopic acuity was poor (800 seconds of arc of disparity) in 6 children and in 18 children (4.1%) no stereopsis was found by Titmus test. Twelve of 24 had been diagnosed and treated because of strabismus (9 cases of amblyopia (3 cases including 2 cases with organic amblyopia) (Table III) 15 of 24 intermittent or small angled alternating strabismus (6 cases) or anisometropia cases) was now discovered and in 3 cases the reason for defective stereopsis remained undetected. Some decrease in stereoscopic acuity (between 800 and 80 seconds of arc) was found in another 57 children (13.9%). Fifteen of them either manifest or latent strabismus (8 cases 4 of them had earlier been treated) decreased visual acuity due to anisometropia or myopia (7 cases 3 of them discovered) and 8 children had mild (less than +2.0 D) hyperopia and symptoms. In 34 of the 57 cases no other abnormalities were detected.

Thirteen of the 19 children with manifest strabismus showed no or poor stereopsis (≤ 800 seconds of arc) in 5 of them stereoscopic acuity was between 800 and 80 seconds of arc and in one child it was normal. The only child with anisometropic amblyopia showed no stereopsis one of the 4 with mild amblyopia had a stereoscopic acuity between 800 and 80 seconds of arc and in 3 children with mild amblyopia no defect in stereopsis was discovered by the Titmus test.

Altogether in 19 cases the causative disorder for defective stereopsis had been known and treated and another 7 children failed at the present screening. The 10 children who had passed the present screening program inter-

Table III
Abnormalities related to defective stereoscopic acuity as found by Titmus screening

Stereoscopic acuity (seconds of arc)	Number of children (%)	Strabismus anisometropia & amblyopia			Total number of children
		Treated earlier	Untreated		
			Failed at present screening	Passed present screening	
≥ 800	24 (5.8%)	17	3	0	3
$< 800 - \geq 80$	57 (13.9%)	7	4	4	4
Total	81	14	7	10	4

* all passed present screening methods

all angled alternating strabismus was discovered but none of them had a metically noticeable deviation. In 3 of the ten wearing of glasses due to peropia and in one case orthoptic therapy due to poor convergence was considered. The others did not require any treatment. The number of false positive ults using the Titmus stereo test (≤ 80 seconds of arc) was 40 or 10.9% of the ole series.

DISCUSSION

ing of the visual acuity by the Snellen chart is still the basic method used in al screening of school children although other methods to detect hyperopia or ors of binocularity have been devised (Sloane 1940, Leverett 1955, Sweeting 59 and others).

The number of children who failed to pass the present screening tests was 17% of the whole series representing the age groups from 7 to 15 years. The 2-percent discrepancy in the figures of the school nurses and the authors when same screening methods were used was mainly due to the difference found in number of children with mild myopia indicating either too short a distance used the examination of the visual acuity in schools or just the peering of children ween half-closed lids. About one fifth of the children who failed at the screening ts did not require any treatment.

Half of the children (9/19) with moderate hyperopia (cycloplegic refraction $+3.0$ or more in one or both eyes) failed to pass the present screening due to reased visual acuity, strabismus or subjective ocular complaints. One quarter re (5/19) would have been detected by the hyperopia test (Table II) for two of se five glasses were prescribed. Wearing of glasses was not considered in derate hyperopia without other abnormalities, strabismus, amblyopia, anisome pia or astigmatism unless subjective symptoms related to reading or other near rk were present. Another quarter (5/19) of hyperopic children remained detected by the hyperopia test. The value of the hyperopia test particularly after age of 8-9 years has been pointed out by Sloane (1940) and Yasuna & Green 52). In this series the use of the hyperopia test would not have added much to present screening results.

In this study the number of amblyopic children was so small that the value of the timus stereo test in screening of amblyopia could not be assessed. However in 3 t of 4 children mild amblyopia would not have been discovered by the Titmus . The shortcomings of the Titmus test in amblyopia screening have also been ported by Simons & Reinecke (1974) and Walraven (1975) although the latter thor stated that in his study on children aged 2 to 7 years the poor performance

of the Titmus test was almost entirely due to its inadequacy for use in 4 year-old children. In the present study the other stereo tests were not used.

Intermittent and small angled alternating strabismus were seldom detected by the present screening methods but all these cases except one were detected by the Titmus stereo test. In this respect a stereo test would have been a supplement to the present methods but the great number of false positives by the Titmus test reduces its value as a screening method. Most of the 22 small angled alternating strabismus detected by the Titmus test did not need treatment because there was no cosmetically noticeable deviation. For vocational guidance the diagnosis of defective stereopsis would have been valuable.

The onset of most visual abnormalities like strabismus (Nordkrantz 1964) or refractive errors occurs before school age and they should also be treated in order to prevent permanent amblyopia or difficulties and delays in the child's performance. Screening for amblyopia should therefore be done at the preschool age as has been advised by Allen (1957, 1967), Lyytikäinen & Koskenvuo (1969), Lippmann (1969), Rosenthal & von Noorden (1971), Tommila (1976) and others. Developing myopia, moderate astigmatism and high hyperopia are the most common causes of decrease in visual acuity. Other refractive errors or slight abnormalities of the visual system seldom need treatment unless subjective symptoms or cosmetically noticeable defects are present. Therefore after proper screening at the preschool age the methods currently used for screening of school children seem to be quite reliable.

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CYSTICERCUS CELLULOSAE IN THE LACRIMAL GLAND ORBIT AND EYE LID

BY

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Six cases of adnexal cysticercosis (1 in the lacrimal gland & orbital and 5 palpebral) have been reported. In the lacrimal gland it presented as a painless translucent cyst which closely simulated a simple dacryops. In the orbit two of the cases presented as an acute abscess in the upper and inner quadrant. However one of them initially presented and existed as a case of simple ptosis for a period of 8 months. The third orbital case appeared as a painless cyst in the upper and outer quadrant. In the lid the larva presented as a subcutaneous nodule in one case and in the other it was lodged in the orbicularis oculi muscle. The cyst was surgically removed in all cases and the diagnosis was confirmed by histopathological examination. Lodgement of cysticercus cyst in the lacrimal gland and in the orbicularis oculi muscle is reported for the first time.

Key words: Cysticercosis - orbital abscess - proptosis - ptosis - lacrimal cyst - nodule - adnexal lesions - orbital cyst

Cysticercus cellulosae the larval form of *Taenia Solium* can get lodged in the ocular tissues but its lodgement in the ocular adnexa is considered rare even in countries where ocular cysticercosis is still common.

The present paper reports 6 cases of adnexal cysticercosis seen in the department of L.N.J.P.N. Hospital New Delhi in detail. The break up of the cases of lodgement is lacrimal gland (1) orbit (3) and lid (2).

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Case Reports

A 14 year-old girl reported with the history of occasional discomfort in the left eye for months. During examination of the eye a translucent smooth cystic swelling about the size of a pea was seen in the palpebral portion of the lacrimal gland. The overlying conjunctiva and the surrounding tissues appeared normal. The cyst was painless on pressure and closely simulated a simple dacryops. The eye ball itself was normal. Ocular rotations were also painless. There was no history of trauma to the region. The cyst was excised completely. Histological appearance of the cyst was typical of a cysticercus cyst (Fig 1).

An 8 year old boy reported with progressive drooping of the left upper eyelid for 6 months. Detailed ophthalmic, general physical and neurological examinations revealed no abnormality. All special investigations were negative. Repeat ophthalmic and medical check-ups at periodic intervals were noncontributory. After 8 months the child was brought with severe pain and swelling in the left eye and fever for 2 days. On examination the left eye was proptosed and displaced downwards and outwards. There was oedema of the lids and periorbital redness and discolouration of the skin on which distended veins could be seen (Fig 2). Ocular movements were grossly limited. The globe itself was normal. A diagnosis of acute orbital cellulitis was made. The cause of cellulitis could not be ascertained. Nose and paranasal sinuses were clinically and radiologically normal. The child was treated with broad spectrum antibiotics and steroid systemically and other supportive measures but suppuration eventually developed. The pus found its way forwards and presented under the conjunctival fornix in the upper nasal quadrant. A free incision was made into the abscess and during drainage a small



Fig 1

Histological appearance of the cyst removed from the palpebral part of the lacrimal gland in case 1 showing the body of the larva *Cysticercus cellulosa*. The membrane around the central cavity shows typical gland shaped convolutions (Haematoxylin and eosin $\times 25$).



Fig 2

Case 2 showing marked forward, downward and outward displacement of the eyelid with oedema of the lids with distended veins on the skin on the left side

translucent cyst with a white spot on its surface escaped along with pus. On cut down it was sterile. The boy recovered with minimal ptosis and some limitation of ocular movement in looking up and in

Case 3 A 6-year-old boy presented with acute orbital abscess in the upper inner quadrant of the left side with signs of optic neuritis. The fundus was normal. During drainage a typical cysticercus cyst escaped. With full doses of broad spectrum antibiotics and systemically the child recovered without any ocular disability.

Case 4 A 16-year-old girl reported for an eye check up. During examination a 6 mm \times 7 mm was discovered in the upper outer quadrant of the left orbit. There was no inflammation around the cyst. The globe was not displaced. Ocular motility was normal. The cyst was completely excised.

Case 5 A 5-year-old boy was brought with a painless subcutaneous nodule 4 mm in size situated just below the middle of left eye brow. It was first noticed 3 months previous. The nodule was excised.

Case 6 A 10-year-old boy accidentally discovered a painless small hard nodule 4 mm in size while passing fingers over the closed left upper lid. During examination it was somewhat mobile under the skin but its mobility was greatly limited when the lids were tightly closed. On surgical exposure the nodule was found embedded in the fibres of the orbicularis oculi muscle. It was completely excised.

None of the cases gave any history of eating pork. A complete general physical examination and multiple tests demonstrated no other foci of cysticercosis in the same eye, in the other eye or anywhere else in the body in any of the cases. The nodule was surgically removed in all the 6 cases and the diagnosis was established by histopathological examination.

Comments

A case with a cysticercus lodged in the lacrimal gland appears to be the first to be reported in the literature. It presented in the form of a painless translucent cyst, which was misdiagnosed clinically as a simple dacryops.

Cysticercosis of the orbit is relatively rare. Out of 372 cases of ophthalmic infection by such cysts recorded in the literature only 19 were found in the orbit (Sjogren 1911) and since then references on orbital cysticercosis have been exceptional. The site of lodgement of a cysticercus cyst is near the orbital margin but only very rarely in the depths of the orbit (Duke Elder 1974). It is of considerable clinical interest to note that a cysticercus was lodged in the depth of the orbit in 2 of the 3 orbital cases reported here. The sequence of events in one of them was unique in that it presented and existed as a case of simple ptosis for which cause could be established for eight months in spite of repeated medical and neurological check ups and extensive investigations.

Cysticercosis of the lid is exceptional (Duke Elder 1974). In recent world literature only one case has been recorded (Jampol et al. 1973). In all cases of lid cysticercosis so far recorded the cyst was found to be lodged in the subcutaneous tissue. One of our lid cases appears to be the first where a cysticercus was found lodged in the orbicularis oculi muscle.

The cases recorded here illustrate that the presentation of adnexal cysticercosis is varied and the condition therefore should be considered in the differential diagnosis of all adnexal lesions in patients from areas where *Taenia Solium* is prevalent.

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Table 1

Summarizing the clinical manifestations in the affected members of the C family

Patient	III 1	III 2	III 6	III 7	III 9	III 3
Age (years)	31	26	16	14	9	50
Sex	m	m	f	f	f	f
Mean spherical error	-18	-7.75	+0.25	+0.25	-2.75	+1.00
Mean astigmatism	2	2.50D	1.75D	1.50D	1.00D	2.50D
Visual acuity	no light sense	6/64	6/60	6/60	6/12	6/60
Macular Change	?	6/60	6/24	6/60	6/36	6/9
Peripheral preretinal membrane	detachment	foveal cyst	degeneration	degeneration	foveal cysts	pigment mottling
Pigmentary degeneration	?	360	270°	270°	-	-
Optic atrophy	?	+	+	+	-	+
Inversion of disc vessels	?	+	+	+	+	+
Vitreous degeneration	+	+	+	-	-	+
Complicated cataract	+	+	-	-	(+)	+
ROC	?	+	-	+	-	+
ERC	?	night blind	flat	?	?	?
Field constriction	?	night blind	flat	?	normal	flat
Squint	my. exom.	+	+	?	?	+
		-	-	lit. gent	ex. h. m.	lit. gent

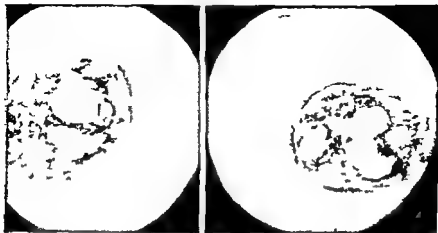


Fig 2

and left maculae in 16 year old girl (III 6) who also has peripheral vitreous hments In the central cystic parts the choriocapillaris and most of the pigment elum are absent The large choroidal vessels are only covered by a thin pellucid slightly ented membrane In the peripheral parts there is heavy pigment epithelium dystrophy ing the choriocapillaris The demarcation towards the periphery is by an annular edematous zone

macular lesions Two types of macular lesions are found in the family members

III year-old propositus has a single cyst in the right fovea and his 9 year-old r has 5 small confluent cysts in both foveae with slight change of the macular ent epithelium A severe bilateral macular destruction is seen in two sisters 16

14 years-old The lesions are two to three disc diameters wide sharply arcated and covered by a continuous membrane which is flush with the ounding retina The central part one to two disc diameters wide is cystic due to absence of the choriocapillaris and most of the pigment epithelium The large roidal vessels and the sclera are covered by a thin pellucid outer membrane and osed to view through the glossy central windows in the inner membrane wards the periphery there is pigment epithelium dystrophy with pigment mulations and depigmentations in some places unmasking the underlying phic choriocapillaris Rarely a retinal vessel crosses the border of the lesion The narcation towards normal retina is by an annular edematous zone

The mother of the siblings has macular pigmentary mottling but there is no cyst mation

peripheral preretinal membranes Peripheral preretinal membranes are present in ee patients The membranes were first observed in the temporal periphery in

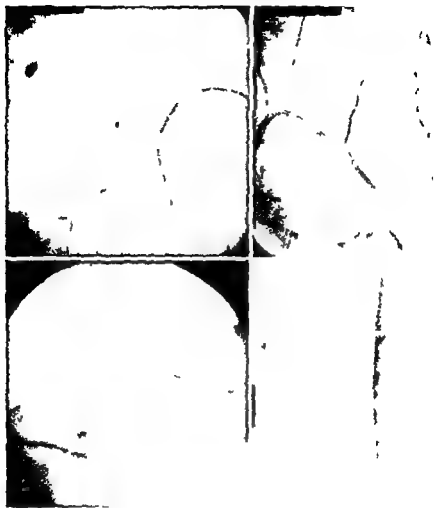


Fig 3

Fig 3a and b show upper temporal preretinal membranes in left and right eyes in a male. The demarcation line is seen tangentially and appears sharp. In a there is condensation of the membrane. In b the lesion has extended across photocoagulation marks. Localized periphlebitis is seen in central vessels. Fig 3c. Ballooning membrane at 12 o'clock in right eye of 16-year-old girl. Cryopexy has been applied. Fig 3d. Nasal periphery of left eye of the same patient showing high preretinal membranes and stretch creases.

front of the equator and gradually extended posteriorly and eventually encompass most or all of the periphery. In two sisters, 16 and 14 years old, membranes originate from the oral region and extend posteriorly to or at the equator. They are ballooning forwards and in one eye actually showing stretch creases on the surface. They are avascular and pellucid but dense enough to

usually seen with the binocular and direct ophthalmoscope. Small condensations are seen in the tri-mirror lens. The posterior attachment of the membrane to the retina is a sharp line although small condensed tongues sometimes stretch further anteriorly. In the 26-year-old proband the preretinal membrane has collapsed along most of the circumference and now presents as a curled up greyish white band near the equator, barely visible in parts; the membrane being only preserved locally temporarily.

Vitreous body. Degeneration of the vitreous is evident in three patients. Two patients have anterior vitreous detachment and anterior fibrillar degeneration and correspond with formation of a few vertical strands. In another patient the vitreous change is less marked with some liquefaction and some syneresis. The two sisters' extensive preretinal membranes apparently have a normal vitreous gel.

Pigmentary degeneration. Apart from the macular lesions pigmentary change is found only in the equatorial region where pigment clumps or a uniform dense hyperpigmentation may be found in the latter case giving a dense brownish appearance.

Papillae. All the affected family members have a uniform disc pallor and in these patients there is inversion of the disc vessels. The retinal vessels generally appear normal apart from occasional localized thinning and periphlebitis in two patients.

Cataracts. Three siblings and the mother have complicated cataracts in the form of posterior subcapsular opacities arranged in a stellate fashion. The oldest blind sister has dense cataracts on both sides.

Refraction and visual acuity. All patients have an astigmatism ranging between one and three dioptres and the mean spherical error ranges between +2 D and -18 D (see Table I).

Ocular tests. Electroretinograms were recorded in three of the patients. In two cases the a- and b-waves were extinguished under photopic and scotopic conditions whereas it was normal in one affected patient. An electrooculogram was only ordered in one case and showed no light rise. One affected patient who was not examined has been complaining of increasing difficulty with night vision for a few years.



Fig 1

Fluorescein angiograms of right macula in 16-year-old girl. Left: Early picture and 10 min. right: Late picture. Note hyperfluorescence in the peripheral parts of the lesion in the late picture. All retention of fluorescein in the demarcation zone corresponds with the annular oedema.

Fig 2

The visual fields When recordable the visual fields in the affected patients show concentric peripheral constriction to around 20° with a large white object blind spot. In one patient with large macular lesions a corresponding central scotoma was also demonstrated.

Motility disorders Two patients have divergent squints and one child has a convergent squint. Two siblings, one of whom without light perception, have horizontal undulating nystagmus.

Fluorescein angiograms were done in three affected patients. In the proband, who has a macular cyst, they show normal masking of the macular choroid and there is no dye leak. In his mother, who has macular pigment change but no exudate or oedema, the 10 min. pictures show diffuse choroidal hyperfluorescence in the fovea. In his 16-year-old sister the late pictures show hyperfluorescence in the peripheral part of the characteristic lesions and in the demarcation zone corresponding with the annular oedema.

General symptoms One patient is severely mentally deficient with an IQ of 56. Two of his sisters are mentally retarded and suffer from spastic hemiplegia. Furthermore, one of these girls and her mother have hypacusis.

DISCUSSION

Four of the six patients included in the material have peripheral preretinal membranes and/or macular lesions. One patient with high myopia developed bilateral retinal detachments in his late teens. It is likely that the etiology for his detachments is the genetic defect apparent in some of his siblings, more especially since it seems that patients with bilateral juvenile retinal detachments are likely to have vitreo-retinal degeneration (Francois et al. 1974). The mother, although having neither central cysts nor peripheral preretinal membranes, displays all the clinical manifestations of the disease and so probably carries the abnormal gene. There is no report of poor eyesight in her family, which would not exclude the disease in a mild form, however. Her husband has normal visual acuity and no vitreous change or retinal degeneration in spite of high myopia.

In the first two subsequent generations and five siblings out of a sibship of nine being affected, the likely mode of transmission is by a single dominant gene with varied clinical expression. A slight possibility would be an autosomal recessive transmission with a high degree of penetrance in the carrier state, whereas a recessive X-linked transmission is ruled out by the fact that both girls and boys carry the disease.

The outstanding signs of the present disorder are the macular lesions and the peripheral membranes. The macular change is seen through several degrees in the affected family members. Three stages can be defined: 1) Early pigmentary change with small pigment granules and depigmentations in the macula, observed in one of the affected girls from the age of three years. 2) Formation of foveal cysts with a preserved choriocapillaris in two of the patients starting around the age of six years. 3) Degeneration of pigment epithelium, choroid and probably retina with formation of a large macular cyst cavity.

The peripheral membranes have only been noticed from an age of eleven years, probably because the patients are difficult to examine. The shape of the membrane in the two girls suggests a retinal splitting, in which case the inner leaf would be made up of the thickened basal lamina of the retina. However, the fact that the retinal vessels in all cases remain at normal retinal level makes it more likely that the membranes are actually thickened posterior vitreous membranes formed after vitreous detachment. At the same time there is probably degeneration of the peripheral retina, as evidenced by the visual field constriction. The extinguishedERG suggests a defect in the receptor cells as well as in the Muller cells.

Differential diagnosis

Signs of hereditary vitreo-retinal degeneration. In this dominant condition the vitreous always shows fibrillar degeneration and a peripheral avascular band or line of Vogt

along the equator. A number of investigators have found a very fine membrane stretching forwards from the equator towards the oral region (1938, Jansen 1962, Alexander & Shea 1965). Hirose et al (1973) demonstrate fusion anteriorly between the membrane and the anterior surface of the degenerate vitreous. They found the vitreous detached both in front of and behind the "grey line". The general consensus is that the membranes are not continuous. Bohringer et al (1960) on section of post mortem eyes of the original Wille family found a membrane attached to the inner limiting membrane of the retina while Ricci (1969) on other sections of the same eyes demonstrated retinal detachment in the nerve fibre layer and macrocystic degeneration of the peripheral retina. Other characteristics of Wagner's disease are localized choroidal atrophy, strands of fibrosis and pigmentations (Alexander & Shea (1965), Jansen (1960)). The macula is always normal. Visual reduction is mainly caused by complicated cataract which requires extraction in the third or fourth decade. There is contraction of visual fields and optic atrophy is common.

The vitreo tapeto-retinal degeneration of Goldmann-Favre. In this rare autosomal recessive condition the patients have early reduction of visual acuity and nyctalopia from early childhood. The morphological characteristics are macrocysts formed by confluence of small cysts and peripheral retinoschisis in the lower temporal quadrant. Scattered pigmentation, sometimes of a corpuscular appearance, and strands of choroidal atrophy are common. There is microfibrillar vitreous degeneration and complicated cataract. The ERG is extinguished in more advanced cases.

In the x-linked juvenile retinoschisis a macular retinoschisis is always present and in the lower temporal vascular retinoschisis is common. Other characteristics are retinal vessels protruding into the vitreous, localized perivascular sheathings, late development of cataract and scattered pigment clumps. Vitreous degeneration is found in advanced cases. Optic atrophy and inversion of the disc vessels occurs. The ERG is extinguished also when there is no peripheral retinoschisis.

The two latter diseases differ from the family condition in question particularly in having peripheral retinoschisis carrying retinal vessels. The other retinal changes in the two diseases are also absent. The differential diagnosis from Wagner's disease is more difficult especially in the proband who has equatorial bands of retinal degeneration and cataract but who does not manifest the other Wagner changes at the posterior poles. His two sisters are clearly distinguished by the presence of macular degeneration which has never been described in Wagner cases or in any of the vitreo-retinal degenerations.

S. A. Hassani: Real time ophthalmic ultrasonography. Springer Verlag New York, Berlin 1978. 214 pages. 423 illustrations and accompanying text and index.

This book deals with the principles of ultrasonography and in particular with ultrasonography in the evaluation of eye diseases.

The book is divided into the following sections: Principles of ultrasonography, eye anatomy, sonography, ultrasonic patient history and oculosonotomography. These give an excellent introduction to ultrasonography.

After this the ultrasonographic findings in eye diseases are presented in the following sections: Sonography of the injured eye, lens, vitreous, retina and choroid, optic nerve, ophthalmic tumors and sonography of the eye in systemic disease. To each section there is an abundance of illustrations of high photographic quality. The accompanying text is excellent. The first section deals with artifacts in ultrasonic investigation.

The book is instructive and can be recommended for practicing ophthalmologists and those dealing with ultrasonography.

Th. Hübner

C. Kommerell: Augenbewegungsstörungen. Neurophysiologie und Klinik (Disorders of motility, neurophysiological and clinical aspects). Symposium der Deutschen Ophthalmologischen Gesellschaft von 15 - 17 April 1977 in Freiburg. J. F. Bergmann, München 1978. 346 pages including table of contents, subject index, references, many figures and pictures in black/white. Price per fascicle DM 90.-

The subjects of the symposium comprise the structure, the function and the innervation of the eye muscles, e.g. how this sensorimotoric reflex pathway is affected by squint operations. One of the other main topics of the meeting is focal diagnosis.

The first chapter of the book contains studies on the morphology of the eye muscles, motor units, metabolism, innervation and the connective tissue apparatus. Then there is a chapter about the peripheral oculomotoric system (a survey, slow eye movements and dynamic power). After a chapter about electromyography there is a chapter concerning various aspects of muscular surgery (comparisons between theoretical and clinical, vertical strabismus caused by horizontal muscles, changes of the saccade velocity after muscle surgery, consecutive divergent strabismus, etc.). The second half of the book contains among other things 13 lectures on the supranuclear organization of the oculomotor system, differential diagnostic considerations in patients with disturbances of gaze, combined motility defects. Then follows a chapter with works on eye movements, a further chapter on cerebellar motility disturbances and associated differential diagnostic problems. The last part of the book contains lectures on, among other topics, various types of nystagmus, fixation disturbances and on normal and abnormal fusion movements.

The contents of the book/the meeting are thus made up of works from neurophysiologists, neurologists and ophthalmologists who all belong to the elite, each in his own expert domain. The drawing up of the congress report and pictures, figures and tables is perfect. In spite of the shape of proceedings it is possible to give the book a quality of unity, thus making it comprehensive with much and much new knowledge amongst the over 100 lectures/articles.

F. C.

Editorial W. Hild, J. van Limborgh, R. Ortmann, T. H. Schebler, G. Töndury and E. Wolff: *Advances in Anatomy, Embryology and Cell Biology*, Vol. 54, Part 4, Vogel M. Postnatal Development of the Cat's Retina, 1978, Springer Verlag, Berlin, Heidelberg, New York, 66 pages, 17 figures, 2 tables, Price soft cover DM 33 — US dollars 16.50

The present study is a light and electron microscopical examination of the retinal development of the cat, both qualitatively and quantitatively. Altogether 17 cats have been examined six hours after birth until adult age with the shortest intervals from the first days after birth. The retinal area examined was temporal to the optic disc.

The first half of the study deals with the results of the qualitative morphological examination, and here the development of the individual retinal layers and their individual features are illustrated with excellent relevant electromicroscopical photomicrographs. Special attention is given to the problem concerning the genesis of the photolamellae, the relationship between the pigment epithelium and the receptors, and morphogenetic cell

movements. In the second section, the quantitative results are discussed, comprising both the volumetric measurements of the retinal structures where the point counting technique is used, together with the examination of the thickness of the individual retinal layers at the different stages of development.

The examination concludes that the individual retinal structures have chronologically different phases of differentiation, and for the retina as a whole, there are three stages of development, namely an initial phase during the first postnatal week, a main phase from the end of the first week until 30 days after birth, and a differentiation phase from 30 days until 6 months postnatally.

Overall, the whole examination results, with demonstration of the centripetal maturation of retinal structures, correspond to previous examination results.

The study can be recommended to anyone interested in electron microscopy of the retina and its development.

K. E. Rasmussen

Kriegstein and W. Leydhecker: *Glaucoma update*, International Symposium, Nara, Japan, May 7–11, 1978, Springer Verlag, Berlin, Heidelberg, New York, ISBN 3-540-09350-8, 224 pages, 48 figures, 27 tables, Price DM 48 — US dollars 26.40

One should not hesitate to advise all ophthalmologists to read the symposium to which various glaucoma specialists have contributed. It gives us an impression of the enormous efforts, both financial and mental, displayed within this field. We must admit, however, that none of the numerous studies have succeeded radically in giving us the weapon required to definitely reduce the incidence of the tragical blindness due to senile glaucoma.

Every ophthalmic medical practitioner must know the truths regarding glaucoma and the instructions concerning treatment.

Every operating ophthalmologist must admit that no really satisfactory procedure exists as

yet, and cannot prevent glaucoma-induced blindness, having no adequate control of this disease state. But we can learn to do our best.

In this book we can read about the enormous efforts displayed to solve the problems, and at the same time about our insufficiency.

P. B. andström

C. M. Bleeker et al *Proceedings of the 3rd International Symposium on Orbital Disorders*
Amsterdam September 5-7 1977 Dr W Junk by Publishers The Hague 3s
London 1978 Price Dutch Guilders 175 - US dollars 87.50

The 3rd International Symposium on Orbital Disorders took place in Amsterdam September 1977. The proceedings from this symposium are compiled in this excellent book comprising 546 pages which deal with practically all aspects concerning the orbit and different diseases that can be found in this small area.

During the last few years rapid progress has been made in the development of techniques of examination and the treatment of the diseases of the orbit, and the reader of the articles of this book not only gets an impression of the present possibilities, but also obtains a critical evaluation of these possibilities. The development of CAT-scanning has especially been revolutionary and has added new dimensions to the diagnosis of the diseases of the orbit and our knowledge of the anatomy of the orbit. Several articles in the book are concerned with CAT scanning but there are also articles about other methods of examination such as ultrasound, phlebography and arteriography. Several articles compare the diagnostic value of these different methods of examination.

The diseases of the orbit - inflammations, tumors, endocrine and vascular diseases - are naturally all treated and discussed in several articles. One advance here was the importance of Thyrotropine releasing hormone (TRH) in the diagnosis of endocrine ophthalmoplegia.

Orbital surgery is also discussed in this symposium and there are articles about both lateral and medial decompression and reparative surgery is also represented.

The work of the 3rd International Symposium on Orbital Disorders is especially relevant for the ophthalmologist, neurosurgeon or otologist who is concerned with the treatment of diseases of the orbit. The book is excellent and can be warmly recommended.

S. Farnick

H. J. Kuchle and H. Basse *Taschenbuch der Augenheilkunde* Verlag Hans Huber & Co.
Stuttgart Wien 1978 Price DM 68 -

The second edition of Kuchle and Basse's *Taschenbuch der Augenheilkunde* is a well known pocket book comprising 408 pages in which the 1st edition from 1965 has been brought up-to-date with regard to diagnosis and treatment. The content - divided into 28 chapters - is just as extensive and adequate as that of the best ophthalmological textbook and are illustrated with relevant photographs (six of these pages are in colour), drawings, diagrams and schemata. One can discuss whether or not it is reasonable in a pocket book to include e.g. data on the emmetropic, myopic and hypermetropic eye and ophthalmometry, but in itself the book is also useful for those non-ophthalmologists with an interest in ophthalmological topics. The authors have managed to get much material into pocket book form partly because the language is short and concise and partly because the book is in small print. At the end of the book there is a valuable survey of the most common ophthalmological syndromes.

Apart from the fact that the contents are quite adequate for a pocket book and a valuable reference of this type, the excellent binding and the quality of the paper make it also useful as such and it can therefore be recommended to anyone needing to be well orientated in ophthalmological problems when the opportunity for consulting the library works is not available.

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CELL MEDIATED IMMUNITY TO HERPES VIRUS TYPE 1 PATIENTS WITH RECURRENT CORNEAL HERPES SIMPLEX

BY

HELGA HAMMER and ATTILA DOBOZY

The proportion of T lymphocytes in the peripheral blood their phytohaemagglutinin reactivity and the leucocyte migration inhibitory effect of purified tuberculin protein were found to be equal in 20 recurrent corneal herpes simplex patients and 17 healthy individuals who had never suffered from clinically manifest herpes simplex virus (HSV) infection. Conversely the HSV vaccine inhibited the leucocyte migration in the healthy group but not in the recurrent herpes patients when the clinical symptoms were manifest. In 14 recurrent corneal herpes simplex patients the examinations were repeated after a 3 month symptom free period and in these periods the patients corresponded equally to the healthy group in every respect.

Key words: herpes simplex virus - corneal herpes - cell mediated immunity

Herpes simplex virus (HSV) is a frequent causative agent of recurrent eye disease. The pathology of the primary corneal herpes simplex is well known, however many questions regarding recurrences remain unsolved. Thus the role of immunologic imbalance in the precipitation and healing of recurrent herpes simplex is unknown.

In spite of the fact that the host defence against virus infections is governed by cell mediated immunity only a few investigations of the cellular immune defence in viral infections have been performed and the results of the lymphocyte transformation test, the lymphokine synthesis and the direct lymphocyte cytotoxicity were contradictory (Rosenberg et al 1974, Russel 1973, Starr et al 1975, Thong et al 1975, Wilson et al 1972).

In the present work the T lymphocyte system in general and the specific cell mediated immunity against HSV type 1 in patients suffering from recurrent

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corneal herpes simplex in the active phase of the disease and in the symptom-free interval and of healthy individuals without any history of manifest HSV infection were compared. To estimate the T lymphocyte system in general the phytohemagglutinin (PHA)-reactivity and the leucocyte migration inhibition test with purified tuberculin protein (PPD) were determined. The specific cellular immunity against HSV was also investigated by means of a leucocyte migration test using HSV antigen.

Material and Methods

Patients. Studies were performed on 25 patients suffering from recurrent orofacial herpes simplex (17 males, 8 females, average age 37 years) in whom recurrences had occurred at least 3 (maximum 7) times. Examinations were carried out in the active phase of the disease and were repeated in 14 patients after a 30-day symptom-free period. The data thus obtained were compared with the data of 17 healthy individuals (11 males, 6 females, average age 31 years) who never suffered from recurrent herpes.

E rosette counting was performed according to the method of Laxer et al. (1970) with some modifications. 1.5×10^5 lymphocytes and 5×10^6 washed sheep erythrocytes were taken in a volume of 0.1 ml Hanks solution and 0.05 ml calf serum was added. The cell suspension was centrifuged (5 min, 400 g, 18°C) and incubated for 2 h at 4°C. Subsequently the sediment was suspended by gentle rotation and the proportion of rosette-forming lymphocytes was determined. Three sheep erythrocytes were taken as a minimum to define the rosettes.

The leucocyte migration test and the method of phytohemagglutinin stimulation of the lymphocytes have been described previously (Hammer 1971, 1974). In the leucocyte migration tests PPD and HSV type 1 vaccine (Lupidon® produced by Hermal, Hamburg) respectively were used as antigens. The extent of migration inhibition was expressed by the migration index, which is the ratio of migrations of cells containing the respective antigen of the controls. A migration index lower than 1 was evaluated as positive. The PHA reactivity was estimated from the incorporation of ^3H -thymidine (cpm/ 10^6 lymphocytes).

Results

There was almost complete agreement between the general activity of the lymphocyte system of healthy individuals and of the patients with recurrent orofacial herpes simplex.

simplex the proportion of E rosette forming cells (T lymphocytes) their reactivity and the leucocyte migration inhibitory effect of PPD (Table I) were to be equal. In contrast to this the HSV type 1 vaccine inhibited the leucocyte migration in the healthy individuals but not in the recurrent corneal herpes simplex patients in the manifest period: the average migration index was significantly lower for the healthy individuals than for the recurrent herpes simplex patients ($P < 0.01$).

In 14 recurrent corneal herpes simplex patients the leucocyte migration inhibitory effect of the HSV vaccine was reexamined after a symptom free period of 3 months duration. In 13 of the 14 patients this antigen resulted in a higher leucocyte migration inhibition (lower migration index) in the symptom free period than in the phase when clinical symptoms were manifest (Table II). The difference between migration indices averaged for the active phase of the disease and for the symptom free state is statistically significant ($P < 0.01$).

DISCUSSION

In the last 15 years have witnessed a transition from the purely serological to the comprehensive immunological aspects of HSV infections.

Table I

Proportion of T lymphocytes in the peripheral blood, their phytohaemagglutinin reactivity and the migration inhibitory effect of purified tuberculin protein and herpes virus type 1 vaccine in patients with recurrent corneal herpes simplex and healthy individuals

	n	T cells (%)	PHA reactivity (cpm/ 10^4 lymphocyte)	Migration inhibitory effect of PPD	Migration inhibitory effect of HSV vaccine
Recurrent herpes (manifest phase)	25	67.6 ± 5.7	32309 ± 7571	0.75 ± 0.15	0.95 ± 0.09
Recurrent herpes (symptom free period)	14	68.2 ± 3.5	34035 ± 6334	0.76 ± 0.17	0.80 ± 0.07
Healthy individuals	17	65.4 ± 6.8	31667 ± 6382	0.76 ± 0.13	0.75 ± 0.13

Significantly lower than in healthy individuals ($P < 0.01$).

Table II
Leucocyte migration inhibitory effect of Herpes simplex virus vaccine in patients with recurrent corneal herpes in the active phase of the disease and after a 3 months symptom free period

Patient	Migration index	
	Symptomatic phase	Asymptomatic phase
1	1.04	0.78
2	0.91	0.82
3	0.98	0.77
4	1.03	0.87
5	0.82	0.64
6	1.09	0.91
7	1.02	0.83
8	0.93	0.71
9	0.98	0.80
10	0.90	0.73
11	0.97	0.82
12	0.81	0.83
13	1.03	0.79
14	0.92	0.82
Mean value \pm SD	0.96 ± 0.08	0.80 ± 0.07

The difference is significant ($P < 0.01$)

There is no explanation of universal validity as to why primary HSV are subclinical in most individuals yet can be very severe or recurrent. There is no doubt that the recurrent or clinically severe HSV infections occur frequently in neonates and in immunodeficient or immunosuppressed than in healthy adults (Linnemann et al 1973; Muller et al 1973; Nahata 1976) but an immunodeficiency cannot be demonstrated in the majority of recurrent herpes patients. Virus-specific antibodies can be detected in a titre in the serum of patients suffering from the recurrent HSV infection (Allen & Allen 1957; Shore et al 1974). When skin testing with HSV antigens performed no differences were found in the response of patients with recurrent herpes compared with that of asymptomatic individuals (Russell 1971; Nahata 1966).

the first step in the present work in vitro methods were used to study the T lymphocyte reaction in healthy individuals and in recurrent corneal herpes simplex patients. The proportions of T lymphocytes in the peripheral blood, PHA reactivity and the leucocyte migration inhibitory effect of PPD were the same in the two groups. This proves that a severe cellular immunodeficiency did not exist in the recurrent HSV infection patients. Similarly Wilton et al (1972) did not find any differences when they compared the lymphocyte responses to PHA and other microbial antigens in seropositive patients without recurrences and patients with clinical exacerbations.

In the other part of our experiments the cell mediated immunities against the viral antigen were compared. In the control group and the recurrent corneal herpes simplex patients who had been asymptomatic for at least 3 months the HSV antigen inhibited the leucocyte migration to almost same extent but it did not cause chemotaxis or chemokinetic inhibition when the clinical symptoms were manifest. Thus independently of whether they suffer from the recurrent HSV infection or not the asymptomatic individuals possess cellular immune defence against the virus. This immune defence decreased temporarily at the time when the clinical symptoms were manifest but is restored after these have subsided. On the basis of these results however it is not possible to decide whether this impairment of the host immune response plays a role in the recurrence or whether it represents the immunological consequence of this.

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EFFECT OF TRISODIUM PHOSPHONOFORMATE AND IDOXURIDINE ON EXPERIMENTAL HERPES SIMPLEX KERATITIS IN IMMUNIZED AND NON IMMUNIZED RABBITS

BY

STEFAN ALENUS¹ ULLA LAURENT² and BO ÖBERG³

The effect of trisodium phosphonoformate (PFA) has been compared to that of idoxuridine (IDU) when applied topically in both liquid and ointment preparations on herpetic keratitis in rabbits. Trisodium phosphonoformate had a therapeutic effect but was not as effective as idoxuridine in the vehicles tested. This was seen with both herpes-immunized and non immunized rabbits.

A herpesvirus mutant inducing a PFA resistant DNA polymerase was used to infect rabbit corneas. A comparison of the effect of PFA on the keratitis caused by this resistant mutant and the wild type herpesvirus indicates that the therapeutic effect of PFA on the herpes keratitis was due to an inhibition of herpesvirus DNA polymerase.

Key words: phosphonoformate – idoxuridine – herpes keratitis – rabbits immunized and non immunized – resistant herpesvirus

Trisodium phosphonoformate (PFA) was recently found to be a selective inhibitor of herpesvirus multiplication by inhibiting the viral DNA polymerase (Helgstrand et al 1978; Reno et al 1978) and to have a therapeutic effect on cutaneous herpesvirus infection in guinea pigs (Alenius et al 1978). Since PFA has a low toxicity in cell culture (Stenberg & Larsson 1978) and in animals (Flodh et al 1978) it was considered important to compare it to IDU in an experimental herpes keratitis model in rabbits.

Materials

Animals A total of 80 male white rabbits weighing between 2 and 3 kg were the study. All eyes were controlled before each experiment.

Viruses and cell cultures Herpes simplex virus type 1 (HSV 1) strain C sensitive to PFA and IDU. Strain C 42 VII PFA^r was resistant to PFA as determined by plaque assay on AGMK cells (Eriksson & Öberg 1979).

Drugs Trisodium phosphonoformate (PFA) was synthesized according to (1924). PFA was used as a 3% aqueous solution in distilled water or as a 5% in water with 0.6% methyl hydroxy propyl cellulose (Methocel® F4M) neutral pH and as a 5% ointment in a petrolatum base (19% liquid paraffin, vaseline 5 PFA). The idoxuridine (IDU) used was commercial preparation: IDU solution (Iduridin®) and 0.2% IDU ointment (Iduridin®). As placebo 0.65% Methocel® in saline and petrolatum base were used.

Methods

Immunization Rabbits were immunized by injecting 0.1 ml of HSV 1 subcutaneously in both ears. Three weeks later the rabbits had a complement titer of 1/8 to 1/32 and were then used for the keratitis experiments.

Inoculation One circular epithelial abrasion was made in the central cornea of each animal by touching it with a 5 mm corneal trephine set to a depth of 0.05 mm. The eyes were then inoculated with 30 µl of HSV 1. The titer of strain C 42 VII PFA^r was 10^7 plaque forming units per ml.

Examination and grading All eyes were examined by the same examiner performed before and after staining with fluorescein using a slit lamp. The examiner did not know which drugs the eyes had received. The epithelial keratitis was graded on a scale of 0 to 3 as follows:

Epithelial score Grade 0: no detectable dendritic ulcer. Grade 0.5: 1 to 4 dendritic ulcers limited to the epithelium along the line of abrasion or dendritic ulcers limited to the line of abrasion. Grade 1: 5 to more dendritic ulcers along the line of abrasion or 2-4 dendritic ulcers not limited to the line of abrasion. Grade 2: 5 to 9 dendritic ulcers not limited to the line of abrasion. Grade 2.5: more than 9 dendritic ulcers not limited to the line of abrasion or a confluent ulcer not exceeding one third of the surface area of the cornea. Grade 3: a confluent corneal ulcer involving one third of the corneal surface.

Statistics Standard statistical methods Mann Whitney U test and sign test, were used (Snedecor & Cochran 1969)

Treatment and experimental design In non immunized animals PFA was applied topically in one eye and IDU in the other eye of each animal. Placebo-treated control eyes were treated with either saline 0.6% Methocel® or petrolatum base solutions were given as one drop per eye. Ointment (0.1 ml) was applied with a swab in the lower fornix of each eye. Treatment started on day 3 post virus inoculation and continued for 4 days. Treatment of immunized rabbits started at day 3 and continued for 4 days. The effect of 5% PFA ointment on the keratitis caused by strain C-42 was compared to the effect on the keratitis caused by strain C-42 VII PFA. One eye was treated with the 5% PFA ointment and compared to placebo treatment of the other eye on the same animal. The PFA treated eye was considered better if the epithelial lesion grade was at least 0.5 lower than the control or worse if it had at least a grade of 0.5 higher and same if the grades were the same. Treatment started on day 3 post virus inoculation and continued for 4 days. PFA and IDU were also applied to non inoculated eyes to test for toxicity.

Results

Effect of PFA in different vehicles

The reduction in keratitis score after treatment with 3% PFA in water and 0.1% IDU solution is shown in Fig. 1. IDU caused a rapid decrease in score but at days 6 and 10 an increase in keratitis score was seen. IDU had a significantly better effect than PFA on the keratitis score at day 4 ($P<0.01$), 6 ($P<0.01$) and 8 ($P<0.05$). To increase the ocular bioavailability of PFA a solution of 3% PFA in 0.6% Methocel® was used and compared to 0.1% IDU solution. As shown in Fig. 2 PFA reduced the keratitis score on day 4 ($P<0.01$) and 6 ($P<0.01$) when compared to placebo. IDU reduced the keratitis scores more than PFA on day 4 ($P<0.05$) but not on any other day. Even in this experiment an increase in the keratitis score was observed in the IDU treated eyes at days 6 and 10 after inoculation. The results were similar when the effect of a 5% suspension of PFA in a petrolatum base was compared to a 0.2% IDU ointment. Treatment with IDU ointment resulted in a lower keratitis score at days 4 and 8 than that seen with PFA ointment. No toxic effects from PFA or IDU, that is punctate keratopathy, increasing intraocular pressure or conjunctivitis were noted in the vehicles tested.

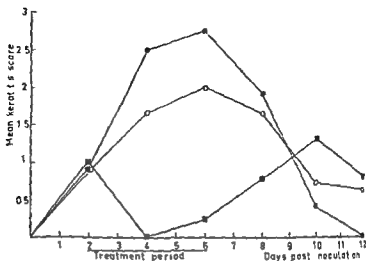


Fig 1

Comparison of PFA water solution and IDU solution in treatment of herpes keratitis. Treatment started 2 days post inoculation all treatments were given every hour during day (12 h) and every second h during the night (12 h) for 4 days. ■—■ 19 eyes were treated with 0.2% IDU solution ○—○ 12 eyes were treated with 3% PFA water solution ●—● 6 eyes were placebo treated with saline

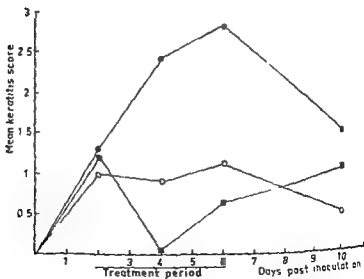


Fig 2

Comparison of PFA as a Methocel® solution and IDU solution in treatment of herpes keratitis. Treatment started 2 days post inoculation all treatments were given every second h during day (12 h) and every second h during the night (12 h) for 4 days. ■—■ 7 eyes were treated with 0.2% IDU solution ○—○ 7 eyes were treated with PFA Methocel solution ●—● 7 eyes were placebo treated with 0.6% Methocel

treatment of herpes keratitis in immunized rabbits

inoculation of HSV 1 C 42 in rabbits immunized with the same strain of virus resulted in an infection with less conjunctivitis and injection than in non immunized animals and without iritis. In these animals the epithelial keratitis developed without total destruction of the cornea which is sometimes observed in non immunized rabbits. The effects of treatment with PFA and IDU were similar to that seen in non immunized rabbits.

treatment of herpes keratitis caused by a PFA resistant virus

The mechanism of the therapeutic effect of PEA was investigated by the use of a herpesvirus mutant resistant to PFA. Table I shows a comparison of the effect on herpes keratitis caused by a sensitive (C 42) and a resistant (C 42 VII PFA) strain of herpesvirus. Each animal was inoculated with the same type of virus in both eyes and the severity of the keratitis was compared within each animal. The average severity of the keratitis caused by the sensitive and the PFA resistant virus was similar on day 3 post inoculation. Treatment with PFA had a better ($P < 0.01$) therapeutic effect on the keratitis caused by the sensitive virus than by the resistant virus.

Table I
Effect of PFA on keratitis caused by sensitive and resistant HSV 1

Days post inoculation	C 42 Condition			C 42 VII PFA Condition		
	B	S	W	B	S	W
3	0	7	1	0	6	2
4	5	2	0	3	4	1
6	7	1	0	4	4	0
8	6	2	0	3	2	3

Treatment started 3 days post inoculation: all treatments were given 5 times daily for 4 days. Eight animals were used for the sensitive (C 42) and eight for the resistant (C 42 VII PFA) HSV 1 strain. The figures show the number of eyes judged as better (B), same (S) or worse (W) when compared to the placebo treated eyes as described in Methods.

Discussion

The therapeutic activity of anti herpesvirus drugs given topically in the eye depend both on the antiviral activity per se and on the concentration reached in corneal cells.

It is likely that after topical application the intracellular concentration of nucleoside analogs (like IDU) which are phosphorylated and trapped in corneal cells will be higher than that of PFA which is not metabolised and probably easily can leave the cell (Flodh et al 1978 Stenberg & Larsson 1978). Even though Methocel® enhanced the activity of PFA IDU still showed a slightly higher therapeutic activity although late during infection a rebound was observed in IDU treated eyes.

As pointed out by Markham et al (1977) it should be of interest to study the antiviral effects of different compounds in an animal model with a strong immune response to herpes simplex virus as this would be closer to the human keratitis. The effect of treatment with IDU and PFA did not differ between immunized and non immunized rabbits.

The PFA resistant herpesvirus mutant used induces a DNA polymerase which is resistant to PFA (Eriksson & Öberg 1979). The lack of therapeutic activity of PFA on a herpes keratitis caused by this resistant mutant (Table 1) indicates that the mechanism of action of PFA on herpes keratitis is an inhibition of the viral DNA polymerase.

PFA did not have a better therapeutic activity than IDU in our experiments which might yet be of clinical interest. The cellular toxicity of PFA has been shown to be lower than that of IDU or ara A (Helgstrand et al 1978). Furthermore the mechanism of action by PFA differs from that of IDU and IDU resistant mutants are sensitive to PFA (unpublished observation).

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ASTIGMATISM AND SURFACE PHENOMENA IN PTERYGIUM

BY

ANDERS HANSEN and MOGENS NORN

Examination of 39 eyes with pterygium and 17 contralateral eyes revealed increased astigmatism with the rule (> 0.5 D in 46% ≥ 4 D in 15%) without associated impairment of vision

Intensified vital staining was seen to be provoked over the pterygium by tetrazolium and rose bengal whereas this was not the case with fluorescein or alcian blue There were no signs of desiccation (Break up time normal)

Stocker's line in front of the pterygium was observed in 46% In 15% this was continuous with Hudson-Stahli's line

A quantitative cytologic study disclosed keratinisation in 5% Increased desquamation was seen in 10%

The central thickness of the cornea was the same as in normal eyes

Keywords: pterygium - astigmatism - direct - BUT - vital staining - Stocker's line - cytology - quantitative

The pathogenesis of pterygium is in the main obscure Ultraviolet light (Cass 1965 Forsius et al 1963 Hogan et al 1967) and perhaps also dryness of the eye are suspected as possible provoking factors The prognosis is unknown Effects towards the centre and development of astigmatism are phenomena to be further investigated

Some workers have noticed astigmatism with the rule and others against the rule (Forsius et al 1962 Bedrossian 1960)

For further elucidation of these matters we decided to summon pterygia patients to be examined for astigmatism and surface phenomena under dryness

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Material

pterygium is a rare defect within our territory i.e. Greater Copenhagen (Vorn 9). We summoned patients from the files collected over a period of several years at hospital and also from ophthalmic practice. The series examined comprised 30 patients of whom two were found to have pseudo-pterygium and accordingly had to be ruled out. (A probe could be passed unimpeded from the upper to the lower edge along the limbus under the large formed mucosal fold). Thus 28 patients were left with a proper pterygium. Of these 11 had pterygium in both eyes and 17 of one eye. In other words there were 39 eyes with pterygium and 17 control eyes without this defect. There were 15 females and 23 males. Only 15 were Danes while 13 were foreigners: the majority Turks and Pakistanis. Their ages ranged from 20 to 80; mean age 52 years.

Methods

Corneal dryness was measured using Javal's keratometer. Values above 0.5 D were characterized as dryness.

Break up time (BUT) was measured initially as described by Vorn (1974). Values below 10 seconds represented pathological dryness.

Slit lamp staining was performed using first a rose bengal fluorescein mixture and some hours later a tetrazolium alcian blue mixture as described by Vorn (1974).

The numbers of stained dots in the individual regions were graded from 1 to 5 corresponding to <30 <100 <1000 <10 000 and >10 000 dots.

Cytologic examinations were based on scrapings from the pterygium and quantitative pipette samples from the inferior fornix of pterygium affected eyes and control eyes.

Table I
Dimensions of pterygium Range ()

	Pterygium N = 39	Unilateral pterygium N = 17
Breadth (mm)	3.42 (0.2-5.0)	2.47 (1.0-5.0)
Height (mm)	4.39 (1.0-7.0)	4.29 (2.0-6.5)
Area (mm ²)	5.78 (0.2-16.2)	9.50 (1.0-16.5)

Presence of more than 50 keratinized nuclear squamous cells in the pterygia indicated keratinisation and presence of more than 50 columnar epithelial cells in desquamation (for details vide Norn 1974)

The central corneal thickness was measured by means of the Haag Streit attachment. Its width area was excluded from the measurement by bringing the double line of the anterior corneal surface to a level line with the corresponding line of the endothelial surface (method A, Norn 1974). Another method employed (method B) was that of measuring using the thinnest possible optical section presumably including the precorneal film.

Result

Site All 39 pterygia were localized nasally never temporally

Dimensions are shown in Table I. In one case the pterygium measured 3.0 mm horizontally from the limbus and in one case 4.0 mm. In the remaining 37 cases under 4.0 mm. No difference was seen between unilateral and bilateral cases. The material comprised both small and rather large pterygia.

Astigmatism was in most cases with the rule both in the pterygium affected eye and in the control eyes with a radius of curvature exceeding 7.5 mm (Table II).

We rarely saw astigmatism against the rule (inverse indirect senile) or oblique astigmatism.

The degree of astigmatism averaged 1.44 dioptres (D) for the 39 pterygium eyes against 0.77 D for the control eyes. The difference is not significant. The highest degree of astigmatism seen was 4.5 D (one pterygium case). No D was observed in

Table II
Astigmatism (> 0.5 D) in % in pterygium affected eye
and contralateral eye

	Pterygium N = 39	Unilateral pterygium N = 17	Control eye N = 17
Astigmatism direct	46	59	47
Astigmatism indirect	10	0	11
Astigmatism oblique	8	6	6
direct ≥ 2 D	11	24	0
direct ≥ 4 D	13	18	0
vision $\leq 6/9$	18	18	18

Table III
Surface phenomena in pterygium in cr

	Pterygium N = 39	Unilateral pterygium N = 17	Control eye N = 17
Stocker's line	46	41	—
Hudson Stahl's line	33	29	18
BUT over pterygium < 10 seconds	91	29	—
BUT whole cornea < 10 seconds	5	12	6

gum affected eyes. Among the control eyes maximum was 1.75 D. At ontal pterygium dimensions of 3 mm or more the astigmatism averaged 1.97 raint 1.11 D in eyes with smaller pterygia. This tendency is not significant r (Mann-Whitney's rank sum test and Student's *t* test).

visual acuity was impaired even with optimum correction owing to cataract tes or senile changes but hardly in any case to pterygium induced astigmatism control eye in Table II).

er's line is a punctate brownish subepithelial line passing vertically in front of nvasive apex of the pterygium. It was present in about half of the cases (Table with no relation to the type of astigmatism, the size of the pterygium, vital ability or BUT.

on Stahl's line is a line passing horizontally below the corneal centre. The line een to have curve shaped contact with Stocker's line in five of the 18 cases in h the latter occurred i.e. in 13% (5/39) of all the eyes with pterygium. e frequency of occurrence of Stahl's line was not significantly higher among ertiarygium affected eyes than among the control eyes.

break up time (BUT) averaged 36.8 seconds in the pterygium affected eyes and seconds in the control eyes. BUT was 42.11 seconds in front of the apex of the ygium. (The figures are minimum figures, the test having been discontinued 60 seconds in 18% of the cases). In no more than two cases was the BUT value over the apex of the pterygium both without Dellen formation. BUT was wise zero in one control eye.

bengal vital staining was significantly more frequent on the surface of the ygium than on the cornea (Table IV, $P < 0.01$). No particularly intense staining noticed in front of the base of the pterygium.

Table II
Vital staining of pterygium remaining cornea and contralateral eye in %

	Pterygium N = 39	Cornea of pterygium affected eye N = 39	Cornea of control eye N = 14
Rose bengal	54	13	24
Fluorescein	64	46	41
Tetrazolum	59	18	12
Alcian blue	18	0	0

Alcian blue rarely stained the pterygium no more than the cornea

Fluorescein vital stained pterygium and cornea to equal extents Staining grade 0.79 on the pterygium and 0.79 on the cornea (control eye 0.76)

Tetrazolum stained the pterygium more frequently (Table IV $P < 0.01$) and intensely (grade 0.97) than the cornea (grade 0.23 control eye 0.24) Of 39 pterygia 10 were stained by tetrazolum alone 8 by rose bengal alone and both dyes

The central corneal thickness was normal in the pterygium affected eye compared with the control eye A systematic difference was noted between the two pachymetry methods employed method B having given an about 20 μm greater thickness than method A

Cytology

Cytologic examination most often gave the same results as in normal eyes

Keratinisation was disclosed in two and increased desquamation in four of 10 quantitative pipette samples One showed signs of bacterial conjunctivitis (many neutrophilic granulocytes) while virus infection was suspected in three (many lymphocytes)

Scrapings from the pterygium showed eight out of 39 to be suspicious for keratinisation (more than 10% nuclear squamous cells had vacuoles or keratin granules)

No correlation was demonstrated between the size of the pterygium and its stainability and pathological cytology

Table 1

Central thickness of cornea of pterygium affected eyes ($N = 39$)
 compared with control eyes ($N = 17$)
 Pachymetric methods A and B vide text

	Method	Cornea thickness (mm)	SEM
Pterygium affected eyes	A	0.483	± 0.007
Pterygium affected eyes	B	0.504	± 0.007
Control eyes	A	0.488	± 0.011
Control eyes	B	0.506	± 0.009

Discussion

ius & Eriksson (1962) found inverse astigmatism to be the most frequent type among their 47 eyes with small pterygia. The inverse astigmatism may however be considered as a senile phenomenon.

In the present series of 39 pterygia of different sizes astigmatism with the rule dominated, being often of a very pronounced degree. Note however that the difference from the control eyes was not significant, presumably because the control series was numerically small (17 eyes).

The results of the present study bear out the view that a pterygium presses against and flattens the cornea, thus increasing the horizontal radius of curvature. Visual acuity remains unaffected, provided the patient is supplied with suitable spectacles.

We have never seen a pterygium extend as far as the centre of the eye, neither in Greenland nor in Copenhagen (Vorn 1979, an additional number of 79 pterygia). In the pterygia of our total series were localized nasally. Exceptions from this rule in the literature may be due to pseudopterygia.

Stocker (1939) believed that the line described by him in front of the base of the pterygium is due to a bend of the cornea at right angles to the flattest corneal meridian. He based his view on two cases observed, of which No. 2 presumably was a case of pseudopterygium, being located infero-temporally on the cornea. The observations made in the series under review do not bear out the theory of a bend. Stocker's line was seen to be continuous with the horizontal Hudson-Stahl's line. Hudson-Stahl's line is present in many normal eyes (Vorn 1968).

Superficial depositings, presumably of iron, are responsible for the occurrence of these two lines. Their sites are probably accountable for by thickened layers of tears. Hudson-Stahl's line corresponding with lacrimal river between the lower lid margin and the

cornea and Stocker's line representing the corresponding layer of tear to the projecting base of the pterygium and the cornea

Barraquer (1965) Mackie (1971) and Paton (1935) hold that dryness of base of the pterygium causes the latter to grow in size

Goldberg (1976) on examination of 59 pterygia by Schirmer's test and found no evidence to support this view

Neither did the present study reveal signs of dryness. The pterygium was certainly often stained by rose bengal but tetrazolium disclosed a similar dense epithelium above the pterygium

Staining by tetrazolium is no indication of dryness (reveals reducing extracellular epithelial cytoplasm). The stainability must be interpreted as an action on surface epithelium of the pterygium whereas not as the cause of growth of pterygium

Fluorescein staining was surprisingly frequent. This was accountable for strain caused by the examination prior to vital staining (eye forced to remain open for a long time during examination for BUT etc.)

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TEAR FLOW AND SOFT CONTACT LENSES

BY

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Tear flow was determined in 14 persons before and after one month of adaptation to a soft contact lens (Softlens® Bausch & Lomb) by means of a radioactive tracer (technetium Tc^{99m} as pertechnetate) a gamma camera and a computer system. The elimination of the radioisotope from the conjunctival sac was diphasic with a significant increase of the fractional turnover rate at the adaptation period in the initial phase with a rapid elimination, but no change in the basal phase with a slower elimination. The fractional turnover rate was also determined with the soft contact lens placed on the cornea after having been pre-soaked in the technetium solution resulting in a value of $0.024 \pm 0.003 \text{ min}^{-1}$ (mean \pm SEM $n = 12$). The fractional turnover rate resulting from instillation of the radioisotope on the non pre-soaked soft contact lens placed in the eye was found to $0.020 \pm 0.004 \text{ min}^{-1}$ (mean \pm SEM $n = 6$). In our studies with the soft contact lens in the eye the elimination curves were monophasic and not diphasic as in normal tear flow studies.

Key words: tear flow – technetium – gamma camera – soft contact lenses – human

In fitting guides for soft contact lenses it is often reckoned that tear deficiency is a relative contraindication for contact lens wear. On the other hand, many authors have reported the results of treatment of dry eyes with soft contact lenses.

A stimulation to the normal human eye will result in an increased tear flow for a few min. In the adaptation period to a contact lens the patients often experience watery eyes for some time. The gradual return to normal conditions can be explained by a fatigue block, as described by Jones (1966). In patients with hard contact lenses the decrease in corneal sensitivity could be responsible for

alization of lacrimation in the adaptation period whereas this can not be the
 nation with soft contact lenses causing almost no changes in corneal sensitivity
 (dot 1974-1976)

The purpose of this paper was to study the possible effect of a well adapted soft
 contact lens on tear flow determined by means of a radioactive tracer and a dynamic
 camera detection system with and without the soft contact lens placed on
 human eye

Material

The material comprised 18 human volunteers - 4 males and 14 females - in the age
 group 20 to 30 years. They were fitted with Sofflens® (Bausch & Lomb) according to
 manufacturer's fitting schedule. All the volunteers were myopic (range -1.50
 to -2.50 dioptres) except one with a low grade of hypermetropia. The patients were
 checked by slit lamp microscopy, ophthalmoscopy and keratometry. Vital staining
 with Rose Bengal and fluorescein was carried out to exclude persons with corneal
 abnormalities. Tear break up time was found normal (over 20 seconds). Most of the
 volunteers joined the group of persons comprising the material of normal persons
 in a tear flow study (Sørensen & Taagehøj Jensen 1979). Four of the patients only
 did the fourth determination.

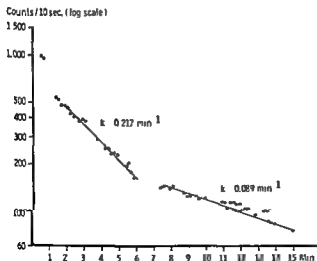


Fig 1

Flow curve from a normal person without contact lenses with an initial phase from 2-6
 min and a basal phase from 7.5-15 min after instillation

Method

The method has been described in detail in another paper (Sørensen & Taagehøj Jensen 1977, 1979). The parameter for tear flow, the fractional turnover, was calculated from the elimination curves of the radioisotope (technetium Tc-99m pertechnetate) from the conjunctival sac. The patients were placed in a supine position with the head fixed under the pinhole collimator in the center of the cornea. A volume of 10 μ l of a normal saline solution with the technetium was instilled into the center of the cornea (dose 100–200 μ Ci). The fractional turnover rate (Fig. 1) was calculated in the initial phase (approximately 2–5 min after instillation) and in the basal phase (7.5–15 min after instillation). Four determinations were carried out in the same eye: 2 before the fitting of the soft contact lens and one after a 2-week adaptation period of at least 3 weeks. This third determination was carried out without a soft lens on the eye immediately after removal of the lens, but with a soft lens on the fellow eye. In the fourth measurement the contact lens was placed on the eye during the recordings. In 12 of these determinations with the soft lens on the eye the lens was pre-soaked in a 10-fold diluted technetium solution for 24 h before the recordings, whereas 6 of the studies were carried out by instilling an undiluted radioisotope solution on the soft contact lens placed on the cornea.

All tear flow determinations were corrected for background radiation as described in a previous paper (Sørensen & Taagehøj Jensen 1979).

Results

The second determination before adaptation was compared to the determination after adaptation (in 2 cases the first determination was used because the first determination failed). As shown in Table 1 the mean fractional turnover rate in the initial phase was found to be higher after adaptation (t test for paired data, $P = 0.05$), whereas the basal fractional turnover rate was of the same magnitude before and after adaptation (t test for paired data, $2P = 20–30\%$). The elimination curves before and after adaptation were all diphasic in a semilogarithmic plot.

When a pre-soaked soft contact lens was placed on the eye, the radioactivity could be seen accumulated corresponding to the position of the soft lens on the cornea (Fig. 2) with some radioactivity entering the lacrimal pathway. A similar distribution was seen when a non pre-soaked lens was placed on the cornea followed by the instillation of 10 μ l technetium solution directly on the soft contact lens. Only small amounts of radioactivity were seen in the conjunctival sac outside the lens in both cases.

Table I

Fractional turnover rate before and after adaptation to a soft contact lens and fractional turnover rates representing the elimination of technetium from soft lenses (mean \pm SE)

	Initial	Basal
Before adaptation (second determination (n = 14))	0.173 \pm 0.015 (min^{-1})	0.076 \pm 0.006 (min^{-1})
After adaptation (third determination (n = 14))	0.091 \pm 0.014 (min^{-1})	0.069 \pm 0.006 (min^{-1})
Soft contact lens pre-soaked in diluted technetium solution (n = 19)	0.091 \pm 0.003 (min^{-1})	
Soft contact lens placed on the cornea during instillation of technetium (n = 16)	0.010 \pm 0.004 (min^{-1})	

With the soft lens placed on the cornea whether pre-soaked or not pre-soaked elimination curves turned out to be monophasic in the semi-logarithmic plot (Fig. 1). The fractional turnover rate was 3-4 times smaller than in the basal phases in tear flow studies without a contact lens in the eye. The difference between the pre-soaked and non pre-soaked group was not statistically significant.

Discussion

Persistent irritation to the conjunctiva has been said to provoke a fatigue block which is thought to be a block in the efferent nerves to conjunctival sensory impulses (Jones 1966). Whether a fatigue block can be caused by a contact lens remains not to be known though the assumption is obvious.

This study showed that the tear flow was not decreased by the adaptation to a soft contact lens. Thus a reduced tear secretion cannot be regarded as a cofactor in the development of deposits on soft contact lenses in normal persons. On the contrary a tendency to a small increase in the initial tear flow was found.

The elimination curves with the soft contact lens placed on the cornea were monophasic with an elimination of approximately 2% per min. This slow monophasic



Fig 2

The distribution of technetium in an eye fitted with a soft contact lens. The radioactivity accumulated in the lens. Radioactivity is also seen in the lacrimal pathways. Three scans with different degree of exposure are shown. Some radioactivity has entered the

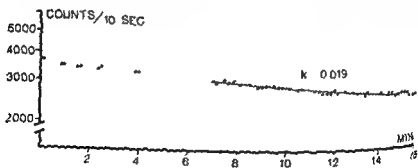


Fig 3

Elimination of technetium from a soft contact lens on a human eye. Radioactivity for conjunctival sac area including the soft lens plotted versus time in a semilogarithmic way. Each dot represents a recording in a ten second interval.

ination was not a result of a fatigue block because the elimination curves in persons wearing hard lenses have a diphasic shape like the curves in normal persons without contact lenses (personal observation to be published later). The explanation may be that the negatively loaded pectinectate was accumulated in the soft contact lens and that the elimination curves represented the elimination of the radioisotope from the contact lens material. The monophasic shape suggested that the elimination curve was dominated by the elimination from the lens making the elimination from the conjunctival sac invisible.

In the pre-soaked and non pre-soaked lenses were found fractional turnover rates of the same magnitude. This means that the technetium after uptake by the lens has been bound in the lens material in a way resulting in an elimination of 1/min when the lens was placed on a human eye.

These results add to the interest of the soft contact lens-drug relationship. It is obvious that some drugs will be retained in the lens material in the similar way as technetium with prolonged corneal contact times. However the elimination of most drugs from soft materials during *in vivo* conditions is unknown and probably depends on the chemical binding between the drug and the lens material.

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THE HLA SYSTEM IN PRIMARY OPEN ANGLE GLAUCOMA AND IN PATIENTS WITH PSEUDOEXFOLIATION OF THE LENS CAPSULE (EXFOLIATION OR FIBRILLOPATHIA EPITHELIOCAPSULAR)

BY

J E SLAGSVOLD and H NORDHAGEN

HLA typing of eighteen antigens in 43 patients with pseudoexfoliation of the lens capsule, 43 patients with capsular glaucoma, and 43 patients with primary open angle glaucoma was performed. No significant deviation from a control series of 45 subjects was found.

Key words: pseudoexfoliation, glaucoma, antigens HLA

Many associations between HLA and disease have been reported especially diseases where an immunological mechanism is suggested (Ryder & Sjö 1976). In ophthalmology the association between HLA B27 and acute anterior uveitis is well known (Brewerton et al 1973) and used as a diagnostic test. HLA compatibility is considered important for the outcome of corneal grafts (Ehlers 1977).

Becker et al (1962) suggested that immune mechanism might be of importance in primary open angle glaucoma. HLA typing of these patients showed an excess of HLA B7 and HLA B12 (Shen & Becker 1976). Increased prevalence of HLA B7 in primary open angle glaucoma was reported by Waltman et al (1975). Others have failed to confirm these observations (Grumet et al 1971, Henley et al 1975, Aviner et al 1976, Grabner & Mayr 1977, Brown et al 1978, Damgaard Jensen & Kissmeyer-Nielsen 1978).

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Scandinavia many studies of pseudoexfoliation and capsular glaucoma have reported discussing sources of the pathological substance and the relationship between pseudoexfoliation capsular glaucoma and primary open angle glaucoma (de 1956 Tarkkanen 1962 Bertelsen et al 1964 Hansen & Sellevold 1968)

As far as we know HLA typing has not been performed in these patients previously. The purpose of this study was to see if HLA typing could give additional information of the relationship between the different groups and may be explain conflicting reports of HLA types in primary open angle glaucoma.

Materials and Methods

Twenty three patients with pseudoexfoliation and normal intraocular pressure (< 19 Hg) and normal optic discs, forty three patients with primary open angle glaucoma and forty five patients with capsular glaucoma (intraocular pressure > 21 Hg, pathological disc cupping and characteristic visual field defects) were

Table I
Frequencies of A-locus and B-locus Antigen Specificities

HLA Antigen	PE N=43	CG N=45	POAG N=43	Controls N=45	χ^2	P_{corr}
A1	11	19	19	11	2.99	
A2	27	25	23	27		
A3	9	11	15*	7		
A9	15	8	5	10		
A10	4	1	2	5		
A11	5	7	6	1	3.97	0.90
A28	1	3	—	—		
B3	—	0	—	3		
B7	14	6	18	9		
B8	0	19*	15	11		
B12	11	18*	7	7	5.54	0.36
B13	—	—	—	1		
B14	1	—	2	1		
B15	15	8	7	13		
B17	—	0	2	3		
B27	6	6	3	4		
Bw35	4	6	3	6		
B40	9	11	8	9		

* test compared to controls

Pseudoexfoliation CG capsular glaucoma POAG primary open angle glaucoma

chosen randomly. Forty five individuals not belonging to any of these groups with minor eye complaints served as controls. Both the patients with primary angle glaucoma and the controls were examined in mydriasis to exclude peripheral foetal material. Pseudoexfoliation represents an old age phenomenon; all patients and controls attending the study had passed 55 years (mean \pm SD). The mean age in each group was: Pseudoexfoliation 71.62, capsular glaucoma 73.51, primary open angle glaucoma 73.31, controls 73.31.

The sex distribution was (F/M): Pseudoexfoliation 33/10, capsular glaucoma 28/17, primary open angle glaucoma 26/17, controls 26/19.

All subjects were from a relatively homogenous population living within the area of the hospital.

HLA typing of 18 antigens with 55 antisera was performed using recombinant microcytotoxicity techniques. All patients and controls were typed blindly at the laboratory knowing the diagnosis. Two-by-two chi square test with continuity correction was used for statistical analyses. Corrected P value (P_{corr}) was found by multiplying the P value with the number of antigens tested for (i.e. 18 (Griffiths 1971)).

Results

The distribution of the HLA A and B antigens in the different groups is shown in Table 1.

The antigen frequencies in the pseudoexfoliation, capsular glaucoma and primary open angle glaucoma groups did not differ significantly from the controls. B12 seems to be increased in patients with capsular glaucoma ($\chi^2 = 5.54$, $P = 0.02$), however $P_{corr} < 0.36$. Likewise there seems to be an increased frequency of B7 in primary open angle glaucoma, but again this is not significant ($\chi^2 = 1.38$, $P = 0.24$, $P_{corr} < 0.90$).

Discussion

In this study we have not been able to demonstrate an association between HLA antigens and primary open angle glaucoma, capsular glaucoma or pseudoexfoliation.

The different results of various investigations of HLA typing in primary open angle glaucoma have been discussed by Damgaard Jensen & Kissmeyer-Nielsen (1978) and Brown et al (1978) taking racial, ethnic, socioeconomical age and diagnostic criteria differences into consideration.

The control population of Shin et al (1977) was racially mixed. No association

made to match patients and controls (Damgaard Jensen & Kissmeyer Nielsen 1978). Furthermore it is not mentioned whether pseudoexfoliation was looked for (diagnosis) in primary open angle glaucoma in any of the studies. Our patients and controls were selected in order to get comparable groups avoiding the above mentioned difficulties although this involved that smaller groups of patients were investigated (materials). However even if we found an increased prevalence of HLA B12 in capsular glaucoma patients and of HLA B7 in primary open angle glaucoma patients P -corvalues showed that the differences were insignificant at the 5% level. Shim et al (1977) did not multiply their P values by number of antigens examined. This would make the increased prevalence of HLA B7 insignificant. Still it is interesting that a trend towards an increased frequency of HLA B7 and B12 was also demonstrated in our material and the close relationship of these antigens in patients with capsular glaucoma and primary open angle glaucoma might call for further investigation. Such a study should preferably include typing for HLA D antigens since associations to disease are more clearly demonstrated with these antigens.

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ULTRASONOGRAPHIC STUDY OF DIABETIC VITREO RETINAL DISEASE WITH LOW VISUAL ACUITY

BY

BENGT JERNELD PEEP ALGVERE and GURSINGH

Ninety three diabetics (168 eyes) with opaque ocular media and low visual acuity (range amaurosis to 0.1) were examined by ultrasonography (A and B scan) using a Coleman Ophthalmoscan 100. Dense vitreous membranes were found in 112 (67%) eyes. 100 (60%) of which showed posterior membranes. Preretinal or prepapillary proliferations (extraretinal stalks) were demonstrated in 71 (49%) eyes. Fifty four (32%) eyes had retinal detachments (40 localized, 14 total). These were present in 10 (50%) of the 20 amaurotic eyes. The ultrasonic accuracy was checked in 49 eyes at vitrectomy. It was 78% for retinal detachments and 67% for prepapillary and preretinal proliferations. The stalks circumscribed with 2 mm or less were the hardest to detect. Ultrasonography thus aids to predict the prognosis after vitrectomy. The visual prognosis appears to be much more optimistic in eyes with vitreous membranes and prepapillary proliferations than in those with vitreous membranes associated with preretinal proliferations.

Key words: ultrasonography · B scan · diabetes · vitreo-retinal disease · vitrectomy

Diabetes mellitus the retinal microangiopathy in itself can cause loss of visual function. However in advanced stages of the disease secondary vitreo-retinal complications such as vitreous haemorrhage and membrane formation, preretinal or prepapillary fibro-vascular proliferations and retinal detachment frequently occur. These sequelae are then largely responsible for the visual failure. Since some of the complications respond to surgery, an ultrasonographic examination using the B scan technique has been advocated when selecting patients for vitrectomy (Coleman & Franzen 1974, Jack et al 1974, McLeod et al 1977).

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In the present work the gross vitreo-retinal pathology was studied by ultrasonography prior to vitrectomy in a number of diabetic eyes with opaque media, low visual acuity. The ultrasonographic examination was focused on the demonstration of some surgically important changes such as dense vitreous membranes, preretinal proliferations and retinal detachment.

Case Material and Methods

During a 20 month period 93 consecutive diabetics (44 males, 49 females) with severe vitreo-retinal sequelae and opaque ocular media were examined. The distribution according to the duration of diabetes is shown in Fig. 1. Patients with visual acuity higher than 0.1 were not included in the study. By the end of the period 49 of the eyes had undergone a pars plana vitrectomy according to the method previously reported (Algvere 1979).

The ultrasonographic study comprised 168 eyes. In 5 patients only one eye was examined. The remaining 13 eyes were excluded due to the presence of corneal disease (or previous enucleation).

Ultrasonography was carried out with a Coleman Ophthalmoscan 100 (Sonotronics Inc., New York, N.Y.) using a 15 MHz focused transducer for A-scan B-scan recordings (Coleman 1972). For general topographic information the

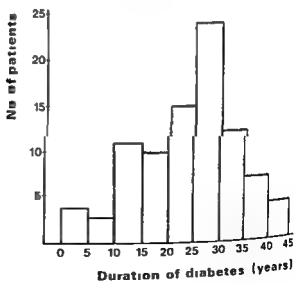


Fig. 1

examined using horizontal B scan sections at 2 mm intervals. Selected areas scanned in detail. When retinal detachments were suspected, even sagittal sections were made. By adjusting the transducer and the eye, high amplitude (perpendicular) echoes from tissue interfaces were sought. The B scan gives an excellent topographic survey but yields no information on the intensity of the echoes. A scan echography on the other hand better reveals interfaces of different acoustic impedance in tissues, and therefore this technique also was employed.

Results

Vitreous membranes The ultrasonic findings were classified into eight categories, two three of which were often present in the same eye (Table 1). Dense vitreous membranes (categories 2-3) were found in the majority of eyes (67%). Posterior vitreous membranes (category 3) were present in 100 (60%) eyes. These membranes were located either close to the retina (epiretinal tissue growth) or were retracted normally into the vitreous space (Fig. 2). In 29 eyes posterior vitreous membranes were the sole ultrasonic finding but in the remaining cases these were associated with fibro-cellular or fibro-vascular proliferations (categories 3 combined with 4-5) and/or retinal detachment (category 6).

Proliferations Totally 71 (42%) eyes displayed preretinal or prepapillary proliferations as extraretinal stalks which were usually associated with vitreous membranes

Table 1
Distribution of 168 eyes according to ultrasonography

Category	Number of eyes	Category	Number of eyes
Vitreous haemorrhage only	8	2 + 4*	1
Central vitreous membranes	9	2 + 5	11
Posterior vitreous membranes	29	3 + 4	14
Preretinal proliferations	6	3 + 5	28
Prepapillary proliferations	4	3 + 6	13
Localized retinal detachment	20	3 + 4 + 5	9
Total retinal detachment	14	3 + 5 + 6	3
Normal ultrasonogram	3	3 + 4 + 5 + 6 + tumour	4
			1

*The combination of two or more categories such as 2 + 4 means that both central vitreous membranes (2) and preretinal proliferations (4) are present.



Fig 2

Ultrasonogram showing dense funnel shaped vitreous membranes (at posterior as detached vitreous) adherent to optic nerve head (to the right) Prepapillary stalk not displayed but was seen at vitrectomy

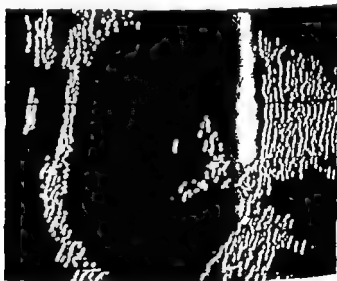


Fig 3

Prepapillary proliferations recognized at vitrectomy as a fibrovascular stalk from optic head



Fig 4

d traction detachment of the retina (arrow) associated with preretinal membranes in posterior pole of fundus Findings verified at vitrectomy

5) As a sole ultrasonic finding such proliferations were thus seen in only 10 (categories 4-5)

cellular or fibro-vascular proliferations arising from the optic disc (category 3) were detected in 50 (30%) eyes (Fig 3) Preretinal proliferations were seen in 10 eyes and in most cases originated from the temporal vascular arcades in the posterior pole

Retinal detachment Fifty four (32%) eyes showed some type of retinal detachment. 14 had a localized detachment (Fig 4) and 40 a total one. In 20 eyes the retinal detachments were associated with ultrasonically dense vitreous membranes (Table categories 3+6 and combinations). Eyes with retinal detachment were classified according to visual acuity. It became evident that the presence or absence of light perception has little if any predictive value for determining the absence or presence of retinal detachment in advanced diabetic vitreo-retinal disease. It was observed that out of the 20 anisotropic eyes 10 had retinal detachments.

Value of ultrasonography The findings at vitrectomy in 49 eyes provided a useful check on the accuracy of preoperative ultrasonography. The preretinal proliferations in 19 and prepapillary proliferations in 22 eyes (found prior to surgery) turned out at vitrectomy to be 27 and 33 respectively. A correct ultrasonic diagnosis having been made in about 2/3 of these cases. In the undetected cases the extraretinal membranes were found at surgery to be associated with posterior vitreous membranes.

Table II
Comparison of findings at vitrectomy and ultrasonography
(49 eyes)

Vitrectomy findings	Ultrasonography observations		
	Vitreous membranes	Retinal detachment	
		Localized	Total
Vitreous membranes	26	1	2
Retinal detachment localized	8	19	

and the proliferations were often circumscribed to small areas (less than 2°) seems that the smaller the stalk the more difficult its differentiation from vitreous membranes ultrasonically.

The most important distinction to be made is that between posterior vitreous membrane and retinal detachment. In this respect the ultrasonic findings were verified at vitrectomy in 38 (78%) eyes (Table II). In the 2 eyes wrongly classified as total retinal detachments the funnel shaped vitreous membranes were such that the echoes recorded were similar to those of a retinal detachment.

Eight eyes showed only vitreous membranes on ultrasonograms but at vitrectomy retinal detachments were found as well. These were flat detachments limited to the posterior pole and associated with thick vitreous membranes. It is pointed out that there was a time lapse between ultrasonography and vitrectomy in these 8 cases: 2 months in one of them and 5-10 months in the others.

Results of vitrectomy in various ultrasonic categories. A correlation was found between the preoperative ultrasonic findings and the vision achieved after vitrectomy. In these eyes the preoperative visual acuity ranged from light projection to 1/60. Post-operative visual increase to 3/60 or more was considered an improvement. Follow-up periods varied between 11 and 24 months (mean 15 months).

Cases with vitreous membranes only and those combined with proliferations showed a postoperative improvement of visual acuity (range 1/60 to 12/60) in 12 of 20 eyes (Table III). The visual prognosis in eyes with proliferations was naturally dependent on the degree of macular involvement. However, it was also substantially affected by the presence of vitreous membranes.

Table III
Visual results of vitrectomy (49 eyes)

Fraction of eyes	Ultrasonographic categories				
	Vitreous membranes	Prepapill prolifer	Preretinal prolifer	Retinal detachment	
				Localized	Total
Improved	5/6	7/14	6/17	4/10	2/9
Unchanged	1/6	3/14	6/17	9/10	
Deteriorated		4/14	5/17	4/10	

Originally diagnosed as retinal detachment surgery disclosed thick posterior vitreous membranes postoperative visual acuity 0.4 and 4/60 respectively

prepapillary proliferations. In all 4 eyes with localized preretinal proliferations at the posterior pole the postoperative visual acuity improved (range 4/60-0.4). On the other hand only 2 of 13 eyes with preretinal proliferations combined with vitreous membranes and/or prepapillary proliferations showed visual improvement (visual acuity 3/60 and 5/60 respectively).

The prognosis in retinal detachment again was influenced by the presence of preretinal proliferations. Of 6 eyes with vitreous membranes and retinal detachment 4 reattached and improved following vitrectomy (visual acuity range 0.2-0.4). 4 eyes with retinal detachment combined with prepapillary and preretinal proliferations ended up with amaurosis (Table III).

Discussion

Vitreo-retinal complications in diabetic proliferative retinopathy are likely to cause severe visual loss. It was reported (Beetham 1963) that approximately 30% of diabetics with proliferative retinopathy were legally blind and that 40% of eyes with such retinopathy had a visual acuity of less than 0.1 (Madsen 1971a).

The present study shows that ultrasonically dense vitreous membranes are seen in 7% of eyes with such low visual acuity. These are comparatively thick structures. The incidence of thin membranes or strands is certainly much higher since only structures of some ultrasonic density are clearly displayed with the method used. In experience even optically dense (yellow-ochre) opacities may escape B-scan

detection. The absence of echoes from long standing vitreous haemorrhage is known also from A scan studies (Oksala 1963).

The preretinal and prepapillary proliferations were associated with the vitreous changes in a high percentage (87%). This again emphasizes the vitreous contraction (Davis 1965) and lends support to Machemers *et al.* (1978) that mechanical factors are involved in the pathogenesis of proliferative retinopathy.

The development of retinal detachment in proliferative retinopathy is a known and crucial event (Tolentino *et al.* 1966, Tasman 1972). The previous retinal detachment in such retinopathy (245 eyes) was reported to be 21% (Lund 1971b). In the present series about 1/3 of the eyes had ultrasonic evidence of retinal detachment. Even if the diagnostic criteria for B scan (Coleman *et al.* 1973) are combined with those for A scan, localized traction detachments of the retina may be difficult to demonstrate. This seems particularly the case where retinal detachments are associated with thick posterior vitreous membrane vitreo-retinal adhesions (Algvere *et al.* 1978). Accordingly our observation that vitrectomy showed retinal detachment in 41% of eyes. Amauric eyes reveal half of the cases studied. Thus with progression of the diabetic disease the incidence of retinal detachment increases considerably.

The accuracy of ultrasonic diagnosis of retinal detachment was 85% in progressive vitreo-retinal disease. The development of such detachment is insidious and may in some eyes have occurred during the time interval between ultrasonography and vitrectomy. If so, our diagnostic error would decrease. In 1974 Jack *et al.* (1974) found an overall accuracy of ultrasound in determining the status to be 85%.

Ultrasonography yields valuable information on the visual prognosis after vitrectomy. Thus 60% of eyes with vitreous membranes and prepapillary proliferations showed postoperative visual improvement (range 0.1-0.9). Preretinal proliferations in the posterior pole when associated with vitreous changes showed improvement in only 2 out of 13 cases. Retinopathy in such cases is generally severe. In addition, preretinal proliferations often have broad vitreo-retinal adhesions and are difficult to excise and when persistent cause tangential retinal traction and detachment.

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THE VARIABLE ANGLE MIRROR A NEW TOOL FOR THE STUDY OF OCULAR DOMINANCE AND EYE FIXATION

BY

ÅKE BJÖRK

A simple tool for studying optical dominance consisting of two plane mirrors moveably fixed to one another was developed. The patient looks straight ahead at the reflection of his own face and observes which eye is covered by the joint between the two mirror halves. By changing the angle a further check-up on eye dominance can be obtained. The method is simple and gives us a qualitative idea of the eye dominance.

Key words Binocular vision — dominance tests — ocular dominance strabismus

Most people are right handed and right footed. This function of limbs is controlled by the contralateral cerebral hemisphere. The eye is different. The partial decussation of the optic fibres at the chiasm links each eye with both hemispheres. We know however that some sort of ocular dominance really exists in a smaller greater part of the general population and can be tested in various ways (Fick) (Coren & Kaplan 1973, Dawson 1949).

Finding reliable tests to point out the dominant eye is difficult. Many tests are available but many of them test different factors which explains why agreement between tests is often poor. Michaels (1972) has contributed an excellent critical review of the whole field of ocular dominance.

Here is described a new test method that seems to have a decided advantage over conventional tests for ocular dominance.

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Methods and Material

Angle Mirror Test

A variable angle mirror (VAM) consists of two plane glass mirrors attached to another by a hinge along the intersection line. The size of the mirror should be big enough to enable the patient to see the whole of his face. The mirror is placed on a table about 0.5 m in front of the patient like an open book, the two glass-plates forming an angle of 90° . The patient looks straight ahead at his own face keeping his eyes open. He is told to ignore the joint between the two mirror halves, which is visible like a vertical line before the image of the face. It is important that the entire face is equally illuminated, otherwise the result may not be valid. A piece of coloured tape is stuck to each of the patient's ears and sides are referred to as e.g. red or green instead of right and left to avoid confusion. The patient can be asked to correct the angle by looking at his own face and by making small adjustments of the mirror halves. The patient is asked if the vertical line covers his red or his green. By moving his head a bit to the right and then to the left he often has a better chance to observe the dark line. Three different situations are possible. The line is in front of the image of the right eye or of the left eye or the patient observes two lines, one before each eye. The two first conditions point out right-eyedness and left-eyedness respectively (Fig. 1). The patient seeing two lines at the same time



Fig. 1

A left-eyed person's view of herself in the angle-mirror



Fig 2

The same situation as in Fig 1 but after having increased the angle of the mirror



Fig 3

A person's view of herself with the left eye covered



Fig 4

The same situation as in Fig 3 but the angle of the mirror is increased

tes no marked supremacy of either eye he is ambicular. In this group there are several intermediate forms and we should note tendencies to right or left dominance. If the patient declares that he can see two lines one of which is straight and may disappear for a while.

Another method using the mirror — still at the trial stage — is to ask the patient to increase the angle between the mirror halves when looking at his face and continue adjustment until the face seems compressed and only one eye is visible in the mirror (Fig 2). With the mirror still in this position the patient is asked to look in the same direction in the room and then into the mirror again. If the same eye is visible every attempt then it is most probably the non-dominant eye.

There are some problems involving reliability of this last mentioned method that have not yet been fully solved. We hope to tackle these and present the results at a later date.

It is really the image of the dominant half of the face that is suppressed can be illustrated by covering one eye of the subject when using the VAM (Figs 3 and 4).

In order to elucidate the question in which extent the results from the WAM test correspond with a sighting test 28 subjects were tested. The results (Table I) show a good correlation between the two methods.

Subject age and sex	Visual acuity and refraction	Heterophoria (Herschel's variable prism with the Maddox groove 5 m)	Stereovision (Titmus)	VAM test	Sighting test
CM 12 M	RF 10 (-1.50) LE 10 (-1.25)	Exoph 1	n	R	R
ER 20 M	RE 15 (+1.50) LE 125 (+0.0)	0	n	R	R
SK 20 M	RE 15 (-3.25) LE 15 (-3.50)	0	n	R	R
IJ 19 F	RE 15 (+0.50) LE 15 (+0.50)	Exoph 10	slight defect	R	R
CK 34 M	RE 15 (-1.25) LE 10 (-1.75)	Exoph 10	n	R	R
VB 5 F	RE 5/3 LE 5/3	-	n	R	-
SM 25 M	RF 20 (± 0) LE 15 (± 0.4 ph -1.2 cyl 1.0)	Exoph 0.5	slight defect	R	R
SC 16 M	RF 20 (-1.0) LE 20 (-1.5)	0	n	R	R
BA 16 M	RF 1 (± 0.4 ph +0.75 cyl 1.95) LE 1 (± 0.4 ph +0.0 cyl 1.00)	Exoph 1.5	n	R	R
WS 35 M	RF 1 (+0.05) LE 1 (-0.01 +0.35 cyl 1.75)	Exoph 0.5	n	R	R

	LF 15 (+0.75)						
OL 97 F	RF 90 (± 0)						RI
	LE 15 (± 0)						
BO 39 M	RE 195 (± 0)						RI
	LE 15 (-0.95)						
EP 90 M	RE 10 (-7.0)						RL
	LE 125 (-7.25)						
JL 59 F	RE 10 (-3.0)						RL
	LE 10 (-3.0)						
LT 65 M	RF 15 (+1.0) sph - 0.5 cy ($\times 80$)						RI
	LF 15 (+1.0) sph - 0.5 cy ($\times 90$)						
LM 93 F	RF 15 (+0.40)						RI
	LE 15 (+0.5)						
CL 9 M	RE 15 (± 0)						RI
	LE 15 (± 0)						
SL 93 F	RF 15 (-1.75) sph						1
	LF 15 (-1.75) sph - 0.5 cy ($\times 90$)						
JL 35 M	RE 15 (+0.95)						1
	LF 125 (+0.50)						
BF 63 F	RF 15 (+1.0)						R
	LE 10 (+1.25)						
OM 97 M	RF 15 (+0.40)						RI
	LF 15 (+0.40)						
BC 31 F	RE 15 (-0.75)						1
	LF 15 (-0.50)						

R = right dominance 1 = left dominance RI = ambocular RI = right dominance sometimes ambocular
 L = left dominance sometimes ambocular n = normal

Results

One of the results of tests with the VAM method was that quite a few subjects did not show a dominant eye. This raises the question of the differentiating capacity of the method itself. This is why a comparison with a sighting method seemed desirable. Our method is a modification of the so called Visierversuch (Hamberger, Sachsenweger 1969 and others).

Our procedure can briefly be described as follows:

The patient is told to keep both eyes open and hold a ruler with both hands and bring it into alignment with a distant object. To perform this operation the patient uses both eyes, not being conscious of which eye he has chosen. By placing a card in front of the patient's right eye and then in front of his left eye we can quickly get an answer which eye is the sighting eye. If the subject for example has used the right eye for taking aim he is right dominant or ambicular. Covering the right eye now forces the subject to aim with his left eye. If on uncovering the right eye the left can maintain the alignment he is ambicular. If however the right eye takes over the subject is right-dominant. This is repeated three times.

At this stage it can be mentioned that the agreement between the VAM test and the above described sighting method is excellent if adequate subjects can be found. Quantitative results will be presented in a future article (see also Table 1).

Discussion

The VAM test is a new, simple method for determining the dominant eye. It has obvious advantages. The subjects often feel motivated to a great extent, and the subject's own face is something which compels attention from other observers, even uncommunicative patients. The mirror can therefore be used in cases where other methods might fail, as with children. The fact that the subjects are ignorant of the purpose of the examination is an advantage and moreover they have no interest in taking part in a sighting operation. They themselves cannot judge what is shown in the mirror, thus giving results probably more reliable than those of the sighting method.

The sighting method requires more instruction than the mirror method. Training in similar circumstances, as when aiming a gun, could contribute to a perhaps incorrect opinion on the eyedness. The sighting operation is often uncertain and vacillating.

Swift and accurate establishment of eye-dominance is of clinical interest. Several authors have pointed to various fields of practical application (Sachsenweger and others). Reference has been made to the importance of knowing the dominant eye before glass correction of anisometropia. It is considered that the

■ eye with reduced vision is more troublesome when affecting the dominant

cases of strabismus with very small deviation angle it can be difficult to decide
1 is the fixating eye and whether an alternation exists. The mirror method
1 to be a useful tool in such circumstances as has been shown in some
nary tests in our laboratory.

this connection the question has been raised as to how early in childhood eye
nance is established and to what extent it changes during the period of growth
furthermore how strong a reduction of vision will result in the loss of
nance.

oking into a mirror when writing writing reversed script without using a
r and similar things in relation to the question of eyedominance have long
a popular field with psychologists. It is hoped that the VAM method will
tribute some details of psychological interest.

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PATTERN CONTRAST THRESHOLDS IN LATENT

BY

RICHARD V ABADI

The evaluation of motor and sensory responses in individuals with latent nystagmus is described. Under monocular, binocular and dichoptic conditions subtle differences in interocular contrast sensitivity are demonstrated. A simple afferent model is proposed to account for the observed results.

Key words: latent nystagmus — contrast sensitivity — monocular and dichoptic viewing — EOG

In some individuals it is possible to generate involuntary oscillations by occluding either eye. This condition is known as latent nystagmus, first reported over one hundred years ago by Faucon (1872). The nystagmus is horizontal and jerky in form, with the fast phase directed towards the open eye.

Many studies suggest that the aetiology of the disorder involves strong genetic influences, which appear to exist (Kornhuber 1960). From Anderson (1954), Crone (1954) and Jung & Kornhuber (1964), it may be considered as part of an oculomotor syndrome — concomitant) and an alternating hyperphoria being the most common feature. Conversely, Jung & Kornhuber (1964) state that about 15% of individuals with latent nystagmus also have strabismic nystagmus.

Because of the nature of the nystagmus and the mechanism by which it is evoked (i.e. by covering one eye), the clinician finds it quite natural to assess such fundamentals as monocular visual acuity. The aim of this paper is to describe the detailed investigation

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ional psychophysical techniques. Contrast sensitivity functions (CSF) of the
 or will be examined under monocular dichoptic and binocular viewing
 ons thus revealing the subtle differences in interocular spatial vision
 more the effect of monocular blur and a controlled change in monocular
 el on spatial thresholds will provide a qualitative description of the effect of
 tasmus on the detection of stationary contours.

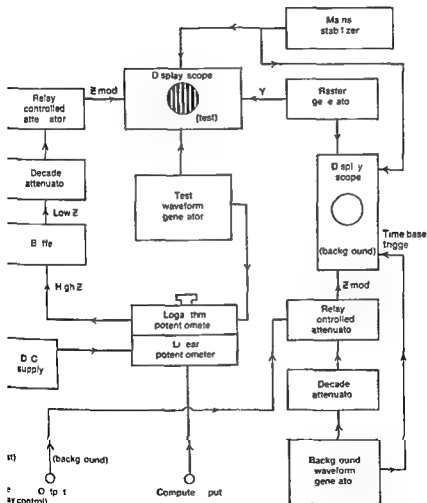


Fig 1

Schematic arrangement illustrating the circuit layout for the generation of the grating pattern

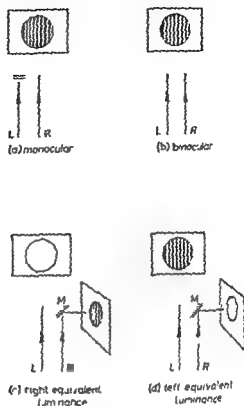


Fig 2

Schematic arrangement of the four different viewing conditions L, R and M for right eye and mirror respectively

Methods

(a) Sensory Tests

Contrast sensitivities were assessed by determining the contrast threshold for sinusoidal gratings of different spatial frequencies (Schade 1976, Campbell & Abadi 1974, Arden 1978). The patterns were generated electronically on the Solartron CD 1400 display oscilloscope (Phosphor P31) and a second mirror provided a second field of an equivalent luminance to that of the first display (Fig 1). In different viewing conditions were used to examine the visual response monocular (Fig 2a) and binocular (Fig 2b) and equivalent luminance (Figs 2c & d).

Contrast thresholds were found by the subjects adjusting a calibrated logarithmic potentiometer until the vertical sinusoidal grating pattern could just be detected. This was then repeated ten times at different spatial frequencies. In this study spatial frequency was defined as the number of cycles per degree of visual angle and contrast as $(L_{max} - L_{min}) / L_{max}$ where L_{max} is the luminance at the centre of the bright bars and L_{min} is the luminance at the centres of the dark bars. The resultant threshold curve may be described as the

ast sensitivity function (CSF) where contrast sensitivity is the reciprocal of contrast threshold. CSFs were determined for all four viewing conditions (Fig. 2a-d).

Effect of a unocular luminance change while the other eye viewed a 2.5 c/deg vertical grating was examined by placing neutral density filters over the left eye. Equal threshold responses were found for each graded increase in monocular retinal illuminance the subjects being initially dark adapted for 20 min.

The circular display field subtended 7° in diameter at a viewing distance of 57 cm. Using a cosine photometer, the pale green displays were calibrated to have a space average luminance of 6 cd/m². Subjects fixated a central spot on the displays during the experiments. Horizontal patterns were used because the direction of the LVN was horizontal and would not degrade the detection sensitivity maximally at this orientation (Abadi & Soglu 1973).

Motor Tests

Monitoring of the direction, amplitude, frequency and velocity of the nystagmus was done by using the electro-oculogram (EOG). The skin was cleaned with acetone prior to application of Cambridge electrode gel and the Ag/AgCl skin electrodes were secured unilaterally about the canthi in the horizontal plane and the reference electrode was placed on the forehead. When the eyes moved a signal was passed through DC amplifiers and a filter (bandwidth up to 30 Hz) and then monitored on a chart recorder. One degree of eye movement was approximately equivalent to 20 µV (Abadi & Sandikcioglu 1974). Recordings were taken both during the experiments and also independently of the psychophysical tasks.

Subjects

Although three subjects were examined in detail, the data from only one of the subjects will be described and commented on, since all three showed similar responses. It is interesting to note that observers S.A. (V.A. 6/24 6/6), G.T. (V.A. 6/6 6/6) and C.S. (V.A. 6/18 + 6/18+) belonged to different visual acuity groups. The primary representative subject for this study was S. (20 years old) in whom LVN was first noticed at 3 years of age and who had no previous treatment or ocular operation in the past. Her present refraction revealed OD -2.25 × 40 6/18+, OS +4.50 -3.50 × 175 6/18+, Binocular V.A. 6/12-.

Di and media normal. Orthoptic examination revealed an alternating hyperphoria as well as a latent nystagmus. On covering one eye, both eyes broke into a conjugate jerky nystagmus with the fast phase towards the open eye and with the eye under cover lagging.

Two control observers were E.E.L., an emmetrope and R.V.A., a corrected myope.

Results

EOG can provide much information about visual processing for they represent a convenient means of describing both the sensitivity and the projected resolution of the visual system. Typical normal curves are illustrated in Fig. 3. Here monocular dichoptic (●) and binocular (○) CSFs show the familiar low (<30 c/deg) and high (>80 c/deg) attenuation in sensitivity. The linear abscissa and logarithmic ordinate correspond to spatial frequency (c/deg) and contrast sensitivity respectively.

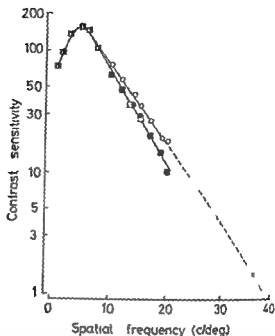


Fig 3

A contrast sensitivity function (CSF) for a normal observer viewing vertical gratings either monocularly (□) right equivalent luminance (●) or binocularly. log/linear co-ordinates the functions may be extrapolated (broken lines) to the resolution limits for the viewing conditions.

ly. With these coordinates the CSF becomes linear from around 8 c/deg. Extrapolated regression lines to unity contrast sensitivity will provide an estimate of the ultimate resolution that may be achieved when viewing a sinusoidal target of 100% contrast. For the control observer E.E.L. the resolution for monocular and dichoptic viewing was 34 c/deg as compared to the somewhat higher value when two eyes were used (39 c/deg).

On the other hand quite dramatic differences are noticed for the subject. As expected the binocular sensitivities are much lower than normal (Fig 4). Interestingly the shape of the whole function is linear with no low frequency attenuation. Genuine indications of monocular vision are illustrated in Fig 5. Here sensitivity responses for dichoptic viewing provide the precise binocular assessments of vision. Right equivalent luminance (Fig 2c and Fig 3) where pattern input enters the right eye only and an equivalent luminance level is provided a curve almost identical to that for binocular viewing (compare Fig 5) whilst left equivalent luminance shows a less sensitive function (Fig 6). The right eye is not only the more dominant but also the more sensitive.

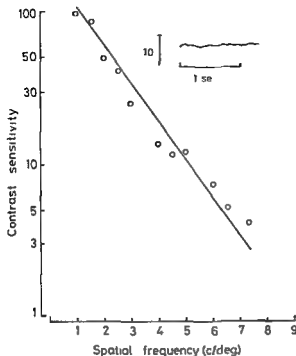


Fig 4

for binocular viewing of vertical gratings. The subject has a latent nystagmus (LN). Inset shows no eye movement response.

traces show no sign of LN under these ideal conditions. If however no care is taken to provide matching background luminance fields and traditional monocular conditions prevail, then a coarse LN comes into play reducing the CSFs for both the right and left eyes (see insets (b) of Figs 5 and 6).

Only one threshold point could be determined for left monocular viewing (Fig 6) due to the large resultant nystagmus on occlusion of the contralateral eye. This contrasts with the uniocular performance of the right eye (Fig 5 -□). Not surprisingly the EOG traces indicate that the induced nystagmus has a greater amplitude and frequency in the less dominant eye.

As far as it has been seen that by providing a dichoptic equivalent luminance arrangement no or little embarrassment of the oculomotor system is achieved. But the highest form of dissociation, monocular occlusion, is attempted disorderly. The question arises as to what factors are responsible for the introduction of nystagmus?

A possible answer to this is expressed in the form of Fig 7. Here the LN is seen

to be induced in a controlled manner by changing the ambient illumination of one eye only thus providing a continuum from monocular to binocular. Moreover the threshold data points are compared with those during binocular and monocular (M) viewing with unilateral blur from a +10 D sphere to assess the effect of head posture and its attendant compensatory conjugate eye movements. By using a low spatial frequency test grating (0.5 c/deg) at low luminance levels the thresholds for the eye under test (i.e. the right eye) within standard errors are unaffected by the very small pupillary changes induced by the neutral density filter's cover of the contralateral eye.

In Fig. 7 the monocular reduction of luminance (abscissa) is plotted against contrast sensitivity (ordinate) for two observers: a control and the observer with a large left eye. As the luminance is reduced in one eye from a factor of 0 to 5 log units, a threshold is measured while the subject views a 2.5 c/deg vertical sine wave. The control observer on progressing from a zero to a 1 log unit change moves from binocular (B) to monocular (M) viewing.

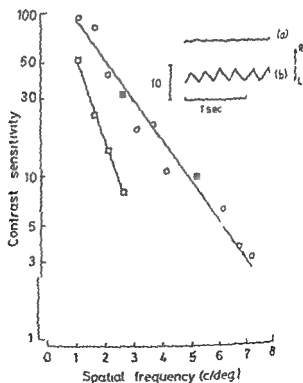


Fig. 5

CSF for right eye equivalent luminance (O) and right eye monocular (□) viewing of vertical sine wave. Subject has a L.A. Inset shows right beating jerky nystagmus only present during monocular task (b) and not during equivalent luminance (a).

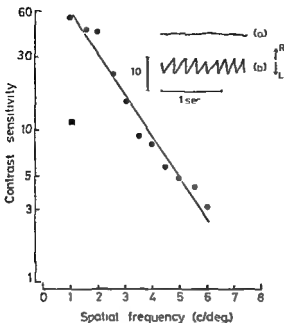


Fig 6

for left equivalent luminance (●) and left monocular (■) viewing of vertical gratings. The inset shows left beating jerky nystagmus only present during left monocular task (b) and not during equivalent luminance (a).

any further monocular luminance reduction has no effect on the contrast sensitivity. A similar relative change also occurs for the latent nystagmat between μ values. However, as the luminance is gradually reduced further sensitivity continues to fall until at a factor of 2.5 log units the sensitivity remains constant. Ratios of the sensitivities for the control versus the nystagmat are given by the open circles (○).

A +10D sphere is placed before the left eye (i.e. equivalent to right eye monocular vision) then the contrast sensitivity is shifted by a factor of 1.0 which is greater than the reduction in sensitivity due to monocular occlusion with a 1.25 log neutral density filter. If the left eye is completely occluded then there is a contrast sensitivity drop of a factor of 5 (Fig 5). However, if the open eye is affected by either converging or turning the head to the right the contrast sensitivity drop is only by a factor of 2 (broken arrow \rightarrow). That is, adduction of the open eye or convergence dampens the nystagmus.

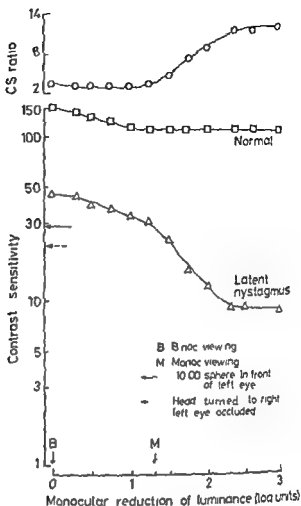


Fig 7

The effect of a unilateral change of luminance on the binocular contrast sensitivity ratio for viewing 2.5 c/deg vertical gratings. Contrast sensitivity (CS) ratio for the two eyes also shown. Observer with LN. Δ Control. \square See text for details.

Discussion

Eye dominance may be expressed in two different experimental modes and sensory. For all three subjects the less dominant eye displayed a intensity (amplitude \times frequency) of nystagmus than its fellow when each target monocularly in turn. The sensory tests yielded the same result in that for the dominant eye showed higher sensitivity values over the whole frequency range. For normal individuals this would not be the case, each approximately equivalent in sensitivity and exhibiting no relative α -oscillation.

is interesting, to note that the shape of the CSF for the nystagmats showed no frequency attenuation. This is most probably due to the induced temporal elements of the retinal image making it less likely that the low frequencies are missed and thus fade (Kulikowski 1971).

Eye movement phenomena are rarely simple to understand and IAN is no exception. Classically IAN occurs during unocular fixation although this present study has shown that if the other eye is viewing an equivalent luminance field then nystagmus will occur. Yet any embarrassment of the binocular state by either retinal blur or a luminance difference induces a jerky nystagmus and a subsequent lowered contrast sensitivity. Moreover neither of these two conditions (i.e. $+10$ D sphere or a 1.2 log unit unocular attenuation of luminance) which effectively transfers vision from a binocular to a monocular state reduces the sensitivity of the system to its true monocular state. Apparently this can only be achieved by ocular occlusion or a large interocular luminance difference — in this case 2.2 until

what then initiates the IAN? Consider that the incoming monocular visual inputs enter a binocular comparator housed in the cortex. If the two signals are equal a neutral result is provided providing no excitement to either of the two monocular control systems i.e. right eye and left eye. If however the two signals are unequal then either a positive (for the right eye) or a negative (for the left eye) signal will be issued thus indicating which of the two should take up fixation since true binocular vision will be inhibited. In normals the inter monocular switching mechanism functions adequately to provide feed back to control the oculomotor system. For the observer with IAN the feed back produces an instability resulting in a jugate drifting of the two eyes. In order to reset the now unbalanced conjugate a rectifying saccade is initiated. Thus the familiar jerky nystagmus results. It must be remembered that although the use of threshold patterns during the haptic experiments did not induce a IAN a high contrast pattern (40%) did induce a IAN in the observer suggesting that the control system must also include some sort of visual threshold device.

Although this argument may be essentially thought of as an afferent theory van der Horst (1973) has proposed that even more central mechanisms may be responsible. Using a most ingenious technique he has been able to describe some cases where subjects who had IAN could induce the jerky oscillations by the intention of looking with one eye. That the patient is required to consciously suppress vision in one eye while fixing with the other is not a particularly difficult task considering that the presence of such anomalies as alternating hyperphorias/tropias and horizontal strabismus are not uncommon in these individuals. Thus the central mechanism reported may be a secondary development to the motor deviations.

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THE OPTICS OF STATIC PHOTOGRAPHIC SKIASCOPY

Comments on a Paper by K. Kaakinen

**A Simple Method for Screening of Children with Strabismus
Anisometropia or Ametropia by Simultaneous Photography of the
Corneal and the Fundus Reflexes**

BY

HOWARD C. HOWLAND

The appearance of reflexes in static photographic skiascopy is a function of myopic or hyperopic focus of the subject relative to the camera-camera to subject distance, subject pupil size, distance of flash relative to optic axis and camera aperture stop. This paper specifies this function.

Key words: static skiascopy - fundus reflex - photography screening of vision defects and eye disorders in young children

Recently Kaakinen (1979) reported a method for the simultaneous photography of corneal and fundus reflexes using a 35 mm camera and flash unit mounted on the periphery of the lens. The technique has the advantage that a great deal of information about eyes is recorded instantaneously in a single photograph. As a method of refraction, however, it leaves something to be desired in that only one meridian of each eye is refracted at a time. Further, for a range of relative refractive errors centered about perfect focusing on the camera, no reflex is seen, and hence nothing can be inferred about the refractive state of the eye except to say that it is within a certain dioptric range of the correct focus. Moreover, this range of relative dioptric defocus is a function of the pupil radius of the subject, r , the distance of the subject from the optic axis passing through the center of the camera aperture and the subject's pupil, h , the radius of the camera aperture itself, a , and the camera to subject distance, d . The purpose of this note is to specify this function.

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Optics of Static Photographic Skiascopy

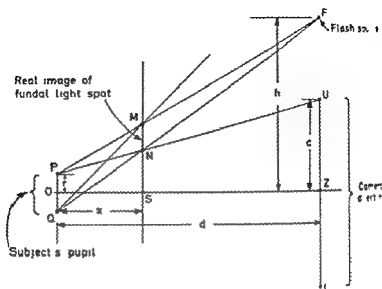


Fig 1

Optics of static photographic skiascopy. The space between the subject and the camera is depicted. The flash is located at point F at a height h above the optic axis passing through the center of the camera lens UZL and the center of the subject's pupil PO . The subject is focused myopically relative to the camera at a distance x from his pupil, where x is the distance from the camera to the subject. A real image of the illuminated spot on the retina is formed at MN since the entering rays FMP and FNQ must also return to the pupil from P to M and Q to N respectively. The horizontal distance of MNS x has been chosen so that the ray through PN will just enter the camera aperture at U causing a crescent to appear on the pupil photograph. The position of x is located by solving the simultaneous equations for the two lines PN and QF and the value of x thus obtained.

Method

In Fig 1 is illustrated the geometry of the static photographic skiascopy. The subject is (myopically) focused on a plane between the camera and himself.

In this situation the out-of-focus image of the flash on the retina must be treated as a point source F in the plane of the camera aperture. It must be so that the subject is focused at a plane in front of the camera at a distance x from his pupil.

At this plane the retinal image is re-imaged in a circle whose diameter in section is MN . Note that each point on MN is illuminated by rays coming

Relative Myopia Necessary for Appearance of Crescent in Static Photographic Skiascopy

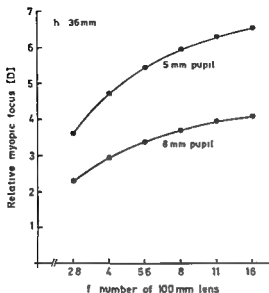


Fig 2

relative myopia necessary for appearance of crescent in static photographic skiascopy. It is noted that the flash source is located 36 mm above the optic axis. The region above each designates the combination of f numbers and relative myopia in which an illuminated crescent will appear in the photograph of the subject's pupil.

Sections of the subject's pupil PQ, but shown are only rays from the extreme edges of the pupil.

If the plane MN is sufficiently close to the subject's pupil, i.e. if the subject is sufficiently myopic, then a ray from the upper edge of the pupil passing to the upper edge of the real image PN may just enter the top of the camera aperture at point U. In this event a crescent will appear in the picture of the subject's pupil. From the diagram it is clear that the subject's pupil radius r , the radius of the camera aperture c , the height of the flash from the optic axis h , and the focus distance of the subject x all play a role in determining whether or not a crescent can be seen. By writing the equations for the lines PNU and QNF in terms of a coordinate system centered at 0 in the subject's pupil POQ and solving for the intersection N of the two lines in terms of x , it is possible to show that a crescent can be seen when

$$x \leq \frac{2rd}{2r + h - c} \quad (1)$$

Similar ray trace diagrams for relative hyperopic focusing by the subject:

$$x \geq \frac{2rd}{c-h+2r}$$

It will be noted that if $(c-h) < 2r$ then the subject must be absolutely hyperopic that a virtual image is formed behind the eye ($x < 0$) before a crescent will be

To illustrate the effect of the size of the camera aperture Fig 9 shows the of relative myopia which the subject must exhibit before a crescent is seen. Pupil photographs for two pupil sizes as a function of f number stop of the camera for a 100 mm lens (suggested by Kaakinen 1979) for a separation of the film from the optic axis. It will be seen that at small pupil sizes and large f numbers the method is quite insensitive to significant degrees of myopia.

Discussion

It is clear that if one wishes to use static skiascopy as a screening method one must carefully set the parameters of the optical system so as to be able to detect the refractive errors in the pupil sizes encountered in the test.

This implies that careful track must be kept both of subject pupil size (which can be measured from photographs) as well as the aperture size used in the camera.

In contrast to the method proposed by Kaakinen (1979) which employs an eccentric flash source refracts a single axis at a time and has a large refractive zone in which little information about refractive error is obtained one may employ a refractive method with a centered fiber optic probe in which multiple axes can be refracted simultaneously and which has a very small refractive depth (Howland & Howland 1974; Howland et al 1978). Admittedly however this method does not permit the simultaneous recording of corneal reflexes.

Acknowledgment

A portion of this work was supported by Grant EY02911-01 from the National Eye Institute. I am greatly indebted to Prof R Weale, Institute for Medical Optics, for allowing me to work in his laboratory on related problems in the optics of photographic refractometry. I thank M Howland for drafting the figures.

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Reply to H C Howland's Comments

BY

K. KAAKINEN

Howland's geometrical analysis of the optics of the static photographic copy is illustrative from a theoretical point of view. However, I would like to emphasize that I have studied the static photographic skiascopy only in practice. The reliability of my method has been tested by Kaakinen (1979) and Kaakinen & Mäkelä (1979) in standard circumstances which are somewhat different from the theoretical analysis of H. C. Howland.

The distance (h) between the optical axis of the camera lens and the center of the pupil is 25 mm instead of 36 mm, and the distance of the lower edge of the flash lamp to the optical axis of the lens is only 15 mm. If 15 mm is used as h , the calculation of the curve then indicates that a minimum myopia refraction of 2.25 diopters (with an 8 mm pupil) would produce a crescent with average value of numbers 11 and 16, which I have constantly used depending on the sensitivity of the film.

However, it should be emphasized that in H. C. Howland's geometrical analysis the flash lamp has been taken as a point source of light. Clearly, the flash lamp is a finite source as far as the corneal reflex is concerned, but H. C. Howland's argument is chiefly with the retinal reflex. In this latter case, exception might be made in treating the flash as a point source, since with respect to the other dimensions used in the geometrical analysis (e.g. camera aperture radius 13-25 mm, pupil diameter 5-8 mm, and distance between light source and optical axis 36

mm 25 or 15 mm) the reflector of the flash lamp is large being 90 mm x 33 mm wide. Moreover to be completely accurate treatment of the flash-light extended source should also take into account the fact that light emission is divergent.

From experiences in practice it is well known that the sensitivity of the method is dependent on the distance of photography and the distance between flash and the optical axis of the camera lens. The influence of the pupillary size on sensitivity of the method for refractive errors is also mentioned in the articles and therefore the photographs have been taken in dim light and atropine medication has also been recommended.

The small influence of the different sizes of the apertures of the camera on the sensitivity of the method for refractive errors is apparent in the photographs of the demonstration eye. Therefore in the clinical studies the aperture numbers 11 and 16 only had been used.

As mentioned in clinical reports it is true that the method is rather insensitive to small refractive errors and only one meridian (vertical) is measured.

However the method can be made more sensitive as desired by using atropine medication or by increasing the distance of photography (d) or by decreasing the distance between the flash light and the optical axis of the camera lens (f) using small aperture numbers (f).

Other meridians can also be measured by turning the flash light to positions on the side of the lens because the method measures the refractive line through the camera lens and the light source. Further the preliminary tests with a partly covered ringflash appear promising in obtaining specific cylindrical astigmatic errors.

The method is most suitable for screening strabismus and high refractive errors. Its accuracy is most sufficient for exact refraction measurements but it needs to be developed further. Worthy of consideration is the fact that the method is very simple for anyone to construct and use. Considering the simplicity of the method it functions surprisingly well in the screening of small children with strabismus, high refractive errors or anisometropia.

There is no doubt about the superiority of the excellent method of Howland (1974) as a refractive technique (US patent 3879113 (A) 618) which multiple axes can be refracted simultaneously with a very small dead zone. But technically the apparatus and the procedure is more complex and the evaluation of results with it is more complex.

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ENUCLEATION IN CHILDREN

BY

INGRID AXELSEN and NIELS EHLERS

In adults enucleation is usually considered a rather simple operation. In contrast the removal of an eye of a child is often a difficult task. We describe an effective technique which makes this surgical intervention a safe and most free procedure.

Keywords: enucleation — children

While the enucleation of an eye from an adult patient usually gives no surgical difficulties, the same intervention in a child may be complicated in many ways.

Psychologically the removal of the eye of a child is always a most unpleasant situation. It may lay a considerable stress on the part of the surgeon, and occasionally interfere with his skill. From the technical point of view the small structures may cause troubles. The sclera may prove thin and the toothed enucleation forceps may lead to perforation with loss of intraocular fluid. In case of a retinoblastoma this is not an acceptable complication.

While cutting the extraocular muscles rarely gives difficulties, the bleeding with a scissor in the retrobulbar tissue in an attempt to cut the optic nerve is to a most stressing haemorrhage. To an ophthalmic surgeon even a small haemorrhage is unusual and he is totally unfamiliar with the amount of a haemorrhage in a small child. Compression is attempted but even if the operation is continued the haemorrhage recommences. After a certain time he may attempt to end the operation by several cuttings with the knife to a bleeding depth. He may realize that the lid aperture is too small to allow it.

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removed and that this is the reason why the eye cannot be pulled out. After a done canthotomy the eye is nervously removed while the bleeding continues. Direct suturing of the conjunctiva is also impeded by the haemorrhage from several unnecessary incisions in the retrobulbar tissue. In the above mentioned complications we find it worth while to report on a simple technique which we have now used for years on a fairly large number of cases.

Recommended technique

An eye speculum is inserted and the conjunctiva is incised along the corneal margin. For this scissors are recommended as a thick and well defined conjunctival flap can be obtained when the subconjunctival tissue is included in the first cut.

A curved pointed scissor is introduced along the globe in each of the four quadrants. By opening the scissor the tissues are separated. With a little experience this can be done only once and causes no haemorrhage.

A muscle hook is placed behind each of the rectus muscles. The conjunctiva is reflected and separated from the muscle. Now the muscle is cut *behind* the muscle leaving 1-2 mm of the muscle tendon on the globe.

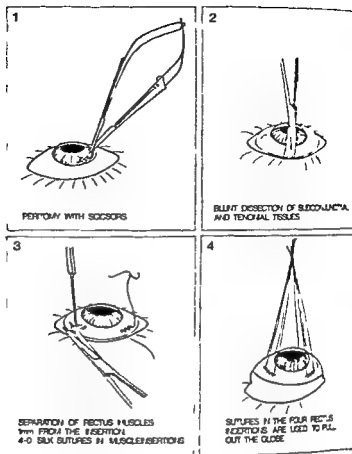
Through each of the four rectus insertions a double 4/0 silk suture is passed twice. A knot is made on the suture in the superior rectus for later fixation.

The four double sutures are twisted and grasped by a pean. It is now easy to pull them forward. The oblique muscles are caught by a muscle hook and cut.

The eye speculum is removed and by pulling on the sutures the eye is gently drawn in front of the lid margins. Now a strong curved scissor is introduced along the medial aspect of the eye. The optic nerve is easily felt and can be cut at any distance from the globe. Only the optic nerve is cut and therefore no or purely minimal haemorrhage occurs.

The conjunctiva is sutured by an absorbable suture e.g. interrupted 5/0 vicryl.

In the case of a retinoblastoma any implant may be considered erroneous as it may prevent the early recognition of orbital recurrence. If no malignancy is present any standard orbital implant can be used and the technique accordingly modified. The operation shows the major steps in the operation (Figs 1-4).



Figs 1-4

Comments

The suggested technique of enucleation has been used in several operations. It has been gradually developed over the years after some unpleasant experiences with the techniques found in many textbooks. It may seem unnecessarily complicated, but it is our experience that the time spent at the beginning of the operation for careful dissection and by placing the traction sutures is fully regained in the later stages.

Besides being a technical improvement it may be advantageous because it is less mechanically traumatic. This might be preferable if tumour spread takes place during mechanical manipulation on the eye. This latter consequence can be understood if tumour cells are found in the anterior chamber but it is not clear if this is with no evident intraocular dissemination of the tumour.

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RETINAL HAEMORRHAGES IN THE NEWBORN

BY

KJELL EGGE GAUTE LYG and JAN MARTIN MALTAU

The present study shows the frequency and severity of retinal haemorrhages in 200 newborn of which 100 were delivered spontaneously, 51 delivered by vacuum extractor and 49 by forceps. The incidence of retinal haemorrhages was highest in the vacuum group (30%) lowest in the forceps group (16%) while the spontaneously delivered children showed an incidence of 41%. The incidence of severe retinal haemorrhages was about five times higher in the vacuum group than in both forceps and control groups. The authors propose a quantitative grading of the haemorrhages. The purpose has been to obtain a better way of relating the haemorrhages to mode of delivery and probably also to relate retinal haemorrhages to possible brain damage.

Key words: retinal haemorrhages, newborn, vacuum extraction, forceps delivery, brain injuries.

Retinal haemorrhages are commonly seen in the newborn during the first few days of life. They can vary considerably from only a single and small one to large and numerous haemorrhages, sometimes also apparently preretinal. Many studies on this topic have been performed, especially during the last 15 to 20 years. The impetus for the present study was that during a short period in the autumn of 1974, two newborn with extremely large and numerous haemorrhages were observed. They were both delivered by vacuum extraction. This study was undertaken to see how the frequency and severity of retinal haemorrhages were influenced by vacuum extraction and forceps delivery in our hospital.

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Fig 1

Retinal haemorrhages in newborn classified as grade I

Materials and Methods

From ultimo September 1974 to ultimo February 1976 ophthalmoscopy was performed in 51 newborn delivered by vacuum extractor and 49 newborn delivered by forceps consecutively. In September 1975 100 consecutive spontaneously delivered newborn were examined in the same way and served as controls. The examination took place during the first 72 hours of life.

The newborn were delivered in the Department of Obstetrics and Gynecology, Rikshospitalet, Oslo. They all had a birth weight of more than 3500 g. Ophthalmoscopy was performed by the direct method using a diagnostic contact lens. Cyclopentolate chloride 1% eye drops (Cyclogyl®) was used as mydriatic. All examinations were performed by the same ophthalmologist.

The retinal changes were classified in three grades (Fig. 1-3).

Grade I was characterized by small, mainly less than a quarter of the optic disc diameter in size, and relatively few haemorrhages in one or both eyes.

Grade II included medium large haemorrhages which were not smaller than the optic disc diameter in size, or a combination of a few such haemorrhages and several smaller ones in one or both eyes.

Grade III included larger haemorrhages, the diameter of which were larger than that of the optic disc, in one or both eyes. Very often the haemorrhages were extremely large, several times the diameter of the optic disc, and were also combined with smaller and/or larger apparently preretinal haemorrhages.



Fig 2

Retinal haemorrhages in newborn classified as grade II

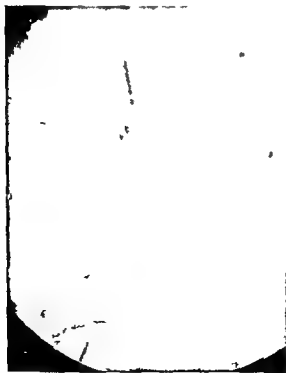


Fig 3

Retinal haemorrhages in newborn classified as grade III

According to studies of von Barsewisch (1979) it is reasonable to assume that many of these so-called preretinal haemorrhages were not preretinal but situated beneath the internal limiting membrane.

Results

Table I shows that

a) the incidence of neonatal retinal haemorrhages was higher in children delivered by vacuum extraction (50%) as compared with those delivered spontaneously (41%)

b) the incidence of retinal haemorrhages in newborn was double those occurring spontaneously and about three times higher in those delivered by vacuum extraction than by forceps

c) the incidence of retinal changes classified as grade III was about equal after vacuum extraction as compared with both forceps or spontaneous delivery

d) what also can be concluded from (c) the incidence of retinal haemorrhages of grade III was about equal in both spontaneous and forceps delivery

Among children delivered by vacuum extractor 16 out of 31 with grade III had these large haemorrhages in both eyes while this was the case of 7 in spontaneously delivered children. All 3 children with grade III delivered by forceps had these alterations in both eyes.

Concerning the haemorrhages of grade I among the spontaneously delivered children 13 out of 20 were affected in only one eye.

Table I
Frequency of retinal haemorrhages in newborn

	Grade III	Grade II	Grade I	Not
Spontaneous delivery (n=100)	7%	14%	20%	
Vacuum extraction (n=31)	34%	8%	8%	
Forceps delivery (n=49)	6%	2%	8%	

Discussion

material in this study is relatively small. However, the observation that the frequency of large retinal haemorrhages is about five times higher after vacuum extraction as compared with forceps or spontaneous deliveries may in our opinion be of considerable importance. We also attach importance to the low incidence of retinal haemorrhages after forceps delivery.

High incidences of retinal haemorrhages after vacuum extraction in relation to spontaneous or forceps delivery have been previously noted by many authors. Sanchez Ibanez et al (1963) found retinal haemorrhages in 59.2% after vacuum extraction, 33.6% after spontaneous delivery and 30.7% after forceps delivery. Kruer Mayer (1965) found 50% after vacuum, 20.5% after spontaneous delivery and 19% after forceps. The corresponding figures reported by Neuweiler & Onwudiwe (1967) were 72.4%, 34.8% and 42.8% while Ehlers et al (1974) found 27.5% and 38% respectively.

The frequency of retinal haemorrhages after vacuum extraction is in this study in agreement with the studies mentioned above in the respect that the incidence in this mode of delivery is considerably higher than after forceps or spontaneous delivery.

In the studies of Sanchez Ibanez et al (1963) and Kruer Mayer (1965) we found the lowest incidence of retinal haemorrhages in the forceps group, while this was not the case in the studies of Neuweiler & Onwudiwe (1967) and Ehlers et al (1974).

Many authors give a description of the appearance and location of the haemorrhages (Brændstrup 1969, Ehlers et al 1974 and others). However, the hitherto most comprehensive description of the different types of haemorrhages is probably given by von Barsewisch (1979) in his monography on neonatal retinal haemorrhages.

To our knowledge, very few attempts have been carried out to grade the haemorrhages quantitatively. Kruer Mayer (1965) points out that after vacuum extraction 15% severe retinal haemorrhages are seen against 2% by forceps and 5% in the control groups. von Barsewisch (1979) has a quantitative subdivision in 5 groups from no retinal haemorrhages in group 1 to more than 20 haemorrhages in group 5, with many visible sources of bleeding and a thrombosis-like structure.

We think, however, that it is better to give a quantitative grading related to the size of the optic disc, completed with a description.

It is reasonable to consider retinal haemorrhages as a complication at birth, despite the high incidence of haemorrhages also seen after apparently normal spontaneous deliveries. The importance of these haemorrhages in the newborn is not clear. Studies of von Noorden & Khodadoust (1973), Lowes et al (1976), Richter

(1976) and others give no basis for an assumption of an increased frequency of amblyopia or squint in patients with retinal haemorrhages at birth. The most difficult question is, however, whether there exists a clear connection between retinal haemorrhages in the newborn and brain damage, for instance in the form of brain dysfunctions. By grading the haemorrhages quantitatively as done above, we hope to give a contribution not only to a better way to relate retinal haemorrhages to mode of delivery, but probably also to relate the haemorrhages to possible brain damage. In our opinion, only long term studies of children, especially those with large and extensive retinal haemorrhages, may give us an answer to this important question.

Acknowledgement

We are grateful to doctor T. Flage for his assistance and help with fundus photography, a venture which caused us a number of inconveniences.

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CHILDHOOD BLINDNESS IN ICELAND

Study of legally blind and partially seeing children in Iceland 1978

BY

SÆVAR HALLDÓRSSON and GUDMUNDUR BJÖRNSSON

In 1978 a study was made of legally blind (corrected visual acuity ≤ 60 or less) and partially seeing (CVA 6/18 to 6/60) children under 15 years of age in Iceland. A total of 43 children were found of whom 23 were legally blind and 20 partially seeing. The prevalence rates expressed as the number per 100 000 children of similar age: 36.4 for legal blindness and 31.1 for partial sight.

In all 43 children the visual loss was attributable to heritable congenital or developmental defects. The most common causes of visual loss were optic nerve atrophy and cataracts. Other causes are listed and discussed. No cases of acquired visual loss were found. In addition to visual loss other congenital birth defects were found in 24 of the children. CNS affection was found in 20 of the children and of these 15 were mentally retarded.

Keywords: blindness – legal blindness – partial sight – congenital blindness – childhood blindness – hereditary blindness

The following study was made in 1978 to determine the prevalence and causes of blindness and partial sight in children in Iceland. While blind children have been studied twice before in Iceland, no study has previously been made of partially seeing children (Sveinsson 1944, Björnsson 1955). Prevalence rates are presented as well as causes and a few case histories are presented briefly.

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Materials and Methods

Information as to the number of blind and partially seeing children, was obtained by polling all practicing ophthalmologists and social workers in the State. Blind Records at the School for the Blind were reviewed and children at the school were examined by the authors. Visual acuity figures and causes of loss are in all cases based on an ophthalmologist's examination. Visual children including all of those with multiple defects were also examined by a pediatrician. Nineteen of the children had been admitted to least to St Joseph's Hospital and in these cases their records were reviewed.

For this study the WHO definition of blindness was used (corrected vision of 6/60 or less in the better eye or a visual field of 20 or less in the better eye). Partial sight is defined as corrected visual acuity from 6/18 to 6/60 in the better eye.

Results

A total of 43 blind and partially seeing children under 15 years of age were examined. Of these 43 children 23 were blind and 20 were partially seeing. Table I shows prevalence rates (Table I) for blindness and partial sight in children under 15 years of age were 36.4 and 31.6 per 100 000 children of comparable age respectively. Distribution of the children by corrected visual acuity is presented in Table II.

Table III shows distribution of the children by site and/or type of eye defect and Table IV shows distribution by etiology. In all cases the visual

Table I
Prevalence rates of legal blindness and partial sight by age

	Population Dec 1 1977	Legal blindness		Partial sight	
		Number	Rate 100 000 population	Number	Rate 100 000 population
0-4	20 142	4	19.8	4	19.8
5-9	20 531	11	53.5	7	34.1
10-14	22 493	8	35.5	9	40.0
	63 166	23	36.4	20	31.6

Table II

Distribution of 43 legally blind or partially seeing children by corrected visual acuity and sex.

Corrected visual acuity	M	F	Total
No light perception	2	1	3
Light perception to finger counting at 1 m	2		2
1/60 to 5/60 Snellen	11	7	18
6/18 to 6/36 Snellen	16	4	20
Total	31	12	43

Table III

Distribution of 43 legally blind or partially seeing children by site and/or type of affection and by age

Site/type of affection	Age			
	1-4	5-9	10-14	Total
Eyeball in General				
Glaucoma		1		1
Anophthalmos and microphthalmos	2	1	2	5
Albinism		6	1	7
Cornea			1	1
Lens				
Cataracts	2	5	4	11
Lens subluxation		1		1
Optic nerve and pathway				
Optic nerve atrophy	3	4	5	12
Nystagmus	1		3	4
Retina				
Macular degeneration			1	1
Total	11	18	17	43

Table II

Distribution of 43 legally blind or partially seeing children by etiology and visual acuity

	Partial sight	Legal blindness	Total
<i>A Hereditary - Congenital</i>			
Albinism - nystagmus		7	
Zonular cataracts	1		1
Down syndrome (Trisomy 21) - cataracts		1	1
Friedreich's ataxia optic atrophy		1	1
Congenital glaucoma	1		1
Microphthalmos anophthalmos		"	"
Monochromatismus (n)stagnus	1		1
Neurofibromatosis optic atrophy		1	1
Smith Lemli-Opitz syndrome lens subluxation		1	1
Spielmeier Vogt's syndrome optic atrophy		1	1
<i>B Other congenital</i>			
Anophthalmos		1	1
Cataracts	7	"	9
Hydrocephalus optic atrophy	2		"
Microphthalmos		"	"
Nystagmus	3		3
Corneal opacity	1		1
Optic atrophy	3	4	7
Macular degeneration	1		1
Total	20	23	43

attributable to hereditary, congenital or developmental defects. The five common causes were optic nerve atrophy, cataracts, albinism and anophthalmos, and together they accounted for 35 (81.3%) of the cases.

The most common cause of visual loss was optic nerve atrophy, which accounted for 27.9% of the cases. Of the 12, 7 were blind and 5 partially seeing. Among the cases born 1976, who had localized cerebral atrophy, but with the exception of one, otherwise normal. A girl born 1968 has Friedreich's ataxia, and her older sister also

sease Optic nerve atrophy began at the age of 4 years in both and they are now blind and severely ataxic. A boy born 1968 had neurofibromatosis like his mother and at the age of 3 optic nerve atrophy was found and attributable to a tumor in the chiasma. The tumor was inoperable but responded well to radiation therapy which did not further impair vision. Myopia accounted for 11 (20.5%) of the cases. Of these 8 are partially seeing and 3 are blind. Of the 9 children with cataracts in category B (Table IV) 9 are blind and 7 partially seeing. All 11 have been operated on for cataracts. Of the 9 2 have nystagmus and 1 has a congenital heart defect. Strabismus accounted for 7 (16.2%) of the cases. All were classified as blind since the best corrected visual acuity was 6/60 or less. They all functioned as well as the partially seeing children and those who were of school age were enrolled in public schools. All have nystagmus and 1 has astigmatism. Five have strabismus and of these four have been operated on. Of the seven are sisters and they have an older albino brother not included in this study. Microphthalmos and anophthalmos accounted for 5 (11.6%) of the cases. All five were blind. A boy born 1967 has microphthalmos, cleft palate and severe mental retardation. A boy born 1968 has anophthalmos, a congenital heart defect (Fallot's tetralogy) and mental retardation. A girl born 1977 has microphthalmos, microcephalus and mental retardation. The family history in all 5 cases was negative. A girl born 1967 had one eye removed at age 2 weeks because of a suspected neoplasm. No neoplasm was found and she has microphthalmos of the other eye. Her brother born 1971 has microphthalmos of one eye and leucoma of the cornea in the other eye. He also has a congenital heart defect and hare lip. The mother has a congenital defect of the lacrimal apparatus.

Of the 43 children 24 (55.8%) have other defects in addition to visual loss. Five have hearing loss and three have congenital heart defects. Twenty have some involvement of the CNS, fifteen of these being mentally retarded. Thirteen have strabismus and seven of these have been operated on. In none of the 43 cases were the parents first cousins.

Discussion

In a study of blindness made in 1940 by Sveinsson found 7 blind children under the age of 20 (Sveinsson 1944). During the National Census of 1950 11 blind children under 20 years of age were found (prevalence rate 15.9 per 100 000 children of comparable age) (Bjornsson 1955). We found 23 blind children under 20 years of age which translates to a prevalence rate of 36.4 per 100 000. We found 17 partially seeing children under 15 years of age for a prevalence rate of 31.6 per 100 000. No previous studies of partial sight have been made in Iceland.

In the present study the cause of visual loss was in all cases attributable to a dietary, congenital or developmental defects. It is known that at least 22 blind children were born in Iceland between 1941 and 1960. Nineteen of these had

congenital cataracts and of these 10 had congenital rubella. Two had atrophy and one had bilateral retinoblastoma (Sigurjonsson 1967). They found no cases of blindness or partial sight attributable to congenital cataracts, which is surprising considering that Iceland had a rubella epidemic in 1963. Following the 1963 epidemic, 37 children with congenital rubella have been examined by the authors. While 57% of the children had retinopathy, none had cataracts or significant visual loss (Baldursson *et al.* 1974). Following the 1972-73 epidemic, a boy was born with congenital rubella, blind with bilateral cataracts, but died during his second year of congenital disease. Another congenital rubella child born 1974 has a unilateral cataract and normal vision in the other eye.

In a study from the United States in 1968, Hatfield found a prevalence of blindness 39.3 per 100 000 among school age children. (Hatfield 1971). A comparable age group in our study had a prevalence rate of 44.1 per 100 000. Causes of visual loss in the American study were intrauterine infections 9.8%, toxic poisonings (including retrolental fibroplasia) 7.9%, tumours 3.7%, trauma 1.4%, prenatal influences 49.9% and unknown 33.6%. (Hatfield 1971). In our study, hereditary, congenital and developmental defects accounted for 18% of the loss in all cases. In the American study they accounted for 49.9% of the loss. The case of RLF has ever been found in Iceland whereas an American study showed that RLF accounted for 8.4% of blindness in children 3-9 years, 14.8% 10-14 years of age and 40.7% 15-19 years of age.

It may also be mentioned that during the present study several children were found who had unilateral visual loss. Causes included congenital rubella, toxoplasmosis, accidents, keratitis, neoplasms, Sturge Weber's syndrome and Goldenhar's syndrome.

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ANGIOTENSIN CONVERTING ENZYME IN UVEITIS AND SARCOIDOSIS

BY

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Serum angiotensin-converting enzyme (SACE) was studied in a group of 100 sarcoidosis patients (among whom five had uveitis) and in 22 patients with non sarcoid uveitis.

SACE was normal in 100 non sarcoid patients and elevated in 69 per cent of the patients with sarcoidosis.

Thus an elevated SACE is closely linked to sarcoidosis and may be a valuable tool in evaluating patients with uveitis in search of a sarcoid origin.

Keywords: angiotensin - converting enzyme - uveitis - sarcoidosis

Approximately half of the patients with uveitis the etiology is unknown (James 1966, Kauffman 1966, Perkins 1976). Uveitis occurs as a complication in a number of infectious disorders and in diseases in which immunological mechanisms seem to play a decisive part, e.g. rheumatoid arthritis, ankylosing spondylitis, sarcoidosis.

Approximately seven per cent of the cases of uveitis are caused by sarcoidosis. In sarcoid disease ocular involvement occurs in 15-20 per cent of the patients submitted to ophthalmological examination (James et al. 1976b, Rømer et al. 1973).

Sarcoid uveitis can be acute or chronic. Frequently affected are females in the age group 20-50 years (James et al. 1976b). The ocular lesion is most often a bilateral iridocyclitis. In acute sarcoid uveitis there is turbidity of the aqueous with few cells and keratic precipitates. In the chronic iridocyclitis nodules on the iris and large keratic precipitates are formed. The posterior uveitis consists of vascular

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sheathing choroidal nodules (waxy exudates or chororetinitis *circa bougie*) (Bruntz 1958) haemorrhages and papilloedema

In recent years serum angiotensin-converting enzyme (kininase II) has been found to be elevated in sarcoidosis (Lieberman 1975)

The present paper is part of a study on SACE in sarcoidosis and granulomatous diseases. The purpose was to examine SACE in 51 sarcoidosis patients with or without uveitis compared with patients with sarcoid uveitis.

Material

A survey of the patients is given in Table I. The series consisted of

1) Twenty-two patients with non-sarcoid uveitis: male/female ratio 11/11 years. The patients were referred to the Eye Department during a 12-month period in 1977/78. The patients with uveitis underwent a standard routine programme including chest X-ray and clinical and laboratory investigation to exclude underlying infectious or rheumatic diseases.

2) Twenty-nine patients with active sarcoidosis: male/female ratio 20/9 years. Sarcoidosis was in most cases diagnosed at the Department of Medicine, the Chest Clinic during 1977/78.

The sarcoidosis patients were staged according to chest X-ray. Stage I: bilateral paratracheal adenopathy. Stage II: hilar or paratracheal lymphadenopathy, pulmonary involvement and Stage III: pulmonary involvement and detectable lymphadenopathy. In 16 out of 29 sarcoidosis patients, non-necrotic granulomas were demonstrated. In 13 patients the diagnosis was made on the

Table I
Survey of the patients included in the study

	No of patients		No of patients
Non-sarcoid uveitis	22	uveitis total	22
Sarcoid uveitis	7	sarcoidosis total	29
Sarcoidosis without uveitis	22		
	51		

ical and roentgenological features and other diseases resembling sarcoidosis (tuberculosis, lymphomas, leishmaniasis, etc.) were ruled out.

In all 51 patients, eight received systemic corticosteroids at the time of the first sampling. Ophthalmic examination included external and slit lamp examination, funduscopy and testing of the vision.

Methods

Angiotensin converting enzyme (SACE)

In all patients, SACE was determined in sarcoidosis patients simultaneously (\pm months) with the ophthalmological examination. In patients with nonsarcoidosis, SACE was determined regularly at their first visit at the ophthalmological department and in most cases repeated after 2-4 weeks. The SACE analysis was performed using the method of Cushman & Cheung (1971) modified by Lieberman (1975). The natural substrate, Angiotensin I, was replaced by a synthetic substrate analogue, the tripeptidyl hippuryl histidyl leucine (Bio Science Products AG, Reusbühl, Switzerland), which SACE liberates hippuric acid. The amount of liberated hippuric acid was measured by spectrophotometry at 228 nm and is a measure of the enzyme activity. SACE is expressed in units, which are equivalent with nanomol hippuric acid formed per min/ml. A 60 min assay at 37°C at pH 8.3 was used.

For the assay, blood was obtained by venipuncture and serum was stored at -20°C until used. After 12 months storage, no loss of enzyme activity was found. The normal values of SACE was 12.0 ± 3.6 units (mean \pm SD) in 116 healthy persons (18-65 years).

Statistics

Differences in mean values were tested with a Student's *t* test. Pair-differences were tested with a Wilcoxon test. Significance level was five per cent.

Results

Sarcoid uveitis

In 29 patients with an abrupt onset and a duration of weeks or months was found in 16 patients (59%) while 9 patients had chronic uveitis with an insidious onset and a duration of months up to years, with several recurrent attacks during the years following admission.

Anterior uveitis (iritis, iridocyclitis) was found in 10 patients (46%), posterior uveitis (retinochoroidal affection and/or vitreous blurring) without affection of the

Table II
Serum angiotensin-converting enzyme (SACE) in 22 patients with non sarcoid uveitis

	No of patients	SACE units (1'0 μ l)	
		Mean \pm SD	Range
Total	22	23.6 \pm 6.8	11-35
With associated disease	5	21.5 \pm 8.4	11-35.5
Without associated disease	17	26.6 \pm 7.9	11.8-35
Anterior uveitis	10	21.8 \pm 6.9	11.7-35.8
Posterior uveitis	8	24.8 \pm 5.8	14.8-35.5
Panuveitis	4	25.7 \pm 6.0	19.4-35
Acute uveitis	13	22.8 \pm 7.3	11.7-35.5
Chronic uveitis	9	24.8 \pm 5.3	14.9-35

anterior segment) was found in 8 patients (36%) and panuveitis (all affected) in 4 patients (18%)

Associated disorders were found in five patients (23%) including Reiter's syndrome, ankylosing spondylitis, unclassified connective tissue disease with proteinuria, glomerulonephritis, rheumatoid arthritis and one case of paraneoplastic uveitis with a protracted course.

As shown in Table II SACE was entirely normal in all patients. No significant difference in SACE was found due to presence or not of associated disorders or to clinical subgroups of uveitis.

Patients with sarcoidosis

SACE was significantly raised compared with non sarcoid uveitis and controls ($P < 0.001$) in 62% (Table III).

No difference in SACE was found according to chest X-ray stage or duration of disease, although the figures in Table III show a trend toward higher SACE and a higher proportion with a chronic course (>2 years).

There was no significant difference in frequency of elevation or in mean SACE between patients with sarcoidosis in whom epithelioid granuloma had been demonstrated (69% SACE 44.4 ± 16.1 (1 SD)) and those where a biopsy had not been taken (54% SACE 41.2 ± 21.1).

In two out of five patients with sarcoid uveitis SACE was elevated (41.1 and 49.1 U respectively). The three other patients were all under prednisone therapy.

The five patients with sarcoid uveitis were 4 females (age 22-56) and one male (age 43). All were bilaterally affected and the uveitis was chronic in all cases.

Table III

Serum angiotensin-converting enzyme (SACE) in 29 patients with sarcoidosis

	No of patients	SACE, units (1° 0-36.8)		No. of patients with elevated SACE	No of patients with uveitis
		Mean \pm SD	Range		
all	29	49.9 \pm 18.2	21.8-102.1	18 (62%)	11 (17%)
at X-ray stage					
	11	38.7 \pm 14.8	21.8-63.2	6 (55%)	2 (18%)
	17	46.8 \pm 19.9	23.0-102.1	12 (71%)	9 (12%)
duration < 2 years	1	24.0	24.0	0	1
duration > 2 years	20	40.7 \pm 18.0	21.8-102.1	11 (55%)	1 (5%)
	9	47.9 \pm 18.7	24.4-84.8	7 (78%)	4 (44%)

interior uveitis with the findings previously mentioned but nodules on iris not seen. One patient had panuveitis with characteristic candle-wax spots in retina. One of the female patients with anterior uveitis had papilloedema in the eye.

during treatment.

total of four patients with sarcoidosis (three with and one without uveitis) were on prednisone treatment at the time of the sampling of blood for analysis. All had normal SACE.

In ten non sarcoid uveitis patients SACE was measured two or four weeks after initiation of prednisone therapy 40-80 mg daily. In two patients a decline of SACE on 20-30% was observed but in the group as a whole no significant decline was found.

Discussion

In recent years serum angiotensin-converting enzyme has been found to be elevated in sarcoidosis (Lieberman 1975) but not in tuberculosis (Lieberman 1975 or 1979) or malignant lymphomas (Rømer 1979). It was the main purpose of this study to investigate the specificity of elevated SACE in a group of patients with clinical and etiological connexion with sarcoidosis i.e. uveitis. In this paper we have demonstrated that non sarcoid uveitis is not followed by elevation of SACE. The mean SACE in 29 sarcoidosis patients was significantly raised in 62% of patients giving a diagnostic specificity (the predictive value of a positive test)

Table II

Serum angiotensin-converting enzyme (SACE) in 22 patients with non-sarcoid uveitis

	No of patients	SACE, units (190 μ l)	
		Mean \pm SD	Range
Total	22	23.6 \pm 6.8	11 - 35
With associated disease	5	21.5 \pm 8.4	11 - 33
Without associated disease	17	26.6 \pm 7.9	11.8 - 35
Anterior uveitis	10	21.8 \pm 6.9	11 - 34
Posterior uveitis	8	24.8 \pm 5.8	11.8 - 33
Panuveitis	4	25.7 \pm 6.0	19.4 - 35
Acute uveitis	13	22.8 \pm 7.3	11 - 33
Chronic uveitis	9	24.8 \pm 5.3	11.8 - 35

anterior segment) was found in 8 patients (36%) and panuveitis (all segments affected) in 4 patients (18%).

Associated disorders were found in five patients (23%) including Reiter's syndrome, ankylosing spondylitis, unclassified connective tissue disease with proteinuria, glomerulonephritis, rheumatoid arthritis and one case of paraneoplastic uveitis with a protracted course.

As shown in Table II, SACE was entirely normal in all patients. No significant difference in SACE was found due to presence or not of associated disorders or to clinical subgroups of uveitis.

Patients with sarcoidosis

SACE was significantly raised compared with non-sarcoid uveitis and controls ($P < 0.001$) in 62% (Table III).

No difference in SACE was found according to chest X-ray stage or duration of disease, although the figures in Table III show a trend toward higher SACE in stage II and a higher proportion with a chronic course (> 2 years).

There was no significant difference in frequency of elevation or in mean SACE between patients with sarcoidosis in whom epithelioid granulomas were demonstrated (69%, SACE 44.4 ± 16.1 (1 SD)) and those where a biopsy was not taken (54%, SACE 41.2 ± 21.1).

In two out of five patients with sarcoid uveitis SACE was elevated (11.2 and 49.1 U respectively). The three other patients were all under prednisone therapy.

The five patients with sarcoid uveitis were 4 females (age 24-57) and one male (age 43). All were bilaterally affected and the uveitis was chronic in all cases.

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of $\frac{18}{18+0} = 1.0$ and a diagnostic sensitivity (the predictive value of a positive test) of $\frac{22}{22+11} = 0.67$ (sarcoidosis vs. non-sarcoid uveitis). Thus normal SACE does not rule out sarcoidosis but elevated SACE points strongly towards sarcoidosis. A series of sarcoidosis patients was too small for splitting up into subgroups but a trend toward higher frequency of elevated SACE was found in patients with chronic disease (duration > 2 years). This trend has been confirmed in a series of 85 patients (Rømer 1979).

Angiotensin-converting enzyme (ACE) converts angiotensin I into the active angiotensin II (Stoffer 1976). It is normally generated in the endothelium, mainly in the lungs. The reason for the elevation of SACE in sarcoidosis is unknown but the finding of a high ACE activity in sarcoid lymph nodes and in ACE formation in epithelioid-cells (Silverstein et al. 1976) and possibly an increased synthesis in these monocyte macrophage derived cells.

In spite of the important role of ACE in formation of angiotensin II, there is no correlation between SACE level and blood pressure. The enzyme also converts bradykinin (kininase II) and it may be that the increased level in sarcoidosis is a change of the kinin metabolism during a low grade granulomatous reaction.

Conclusion

Based on our results we conclude that elevated SACE is a valuable diagnostic test for sarcoidosis and in evaluating uveitis an elevated SACE strongly indicates sarcoid origin.

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eral cell loss can be assessed retrospectively by using the contralateral eye as a control. Recently a low endothelial cell density has been reported in some patients with uveitis (Setälä 1979).

Subjects and Methods

Twenty patients were included in the study. They comprised all patients who had been referred to the eye clinic for the past one and a half years with one to several attacks of acute anterior uveitis. Mean age was 40 years (range 23-69 years). With the exception of two patients who showed a weak seroreaction for toxoplasmosis no underlying etiology to the disease was found in any of the patients. In three patients the anterior uveitis coexisted with a posterior uveitis. During admission all patients were noted to have increased thickness or oedema of the cornea and precipitates were found on the posterior aspect of the endothelium. Patients were not classified according to the type of the precipitates. At the time of referral one patient had elevated intraocular pressure on the affected side while the remainder of the patients had normal pressures (applied).

The shortest time period between discharge from the hospital and the present examination was three months (range 3-18 months). With the exception of three patients who showed a

Table I

Endothelial cell density and central corneal thickness (CCT) in 13 patients with previous unilateral acute anterior uveitis

Patient No	Age	Cell density (cells mm ⁻²)		CCT (mm)	
		Normal eye	Affected eye	Normal eye	Affected eye
1	66	2825	2682	0.510	0.515
2	33	2834	2666	0.520	0.520
3	28	3113	3039	0.520	0.490*)
4	41	2908	2945	0.505	0.495
5	20	3486	3378	0.520	0.545*)
6	61	2703	2759	0.490	0.500
7	35	3146	3281	0.525	0.535
8	49	2778	2330	0.520	0.540)
9	25	2796	2889	0.525	0.530
10	35	3281	2573	0.510	0.540**)
11	69	2796	2852	0.560	0.560
12	42	2288	2306	0.520	0.515
13	44	2950	3202	0.505	0.515

*Healed corneal ulcer on that side

)Faint aqueous flare on that side

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CHANGES IN THE CORNEAL ENDOTHELIUM AFTER ACUTE ANTERIOR UVEITIS AS SEEN WITH THE SPECULAR MICROSCOPE

BY

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In order to investigate whether inflammation of the anterior uveal tract is accompanied by a loss of endothelial cells 13 patients with previous unilateral attacks of anterior uveitis were photographed with the non-contact specular microscope. All patients had shown an increased thickness or frank oedema of the cornea during the acute phase of the inflammation. As compared to the healthy eye only two patients (15%) showed endothelial cell densities on the affected side which were lower than was to be expected from the normal difference between counts from left and right eye in a control population of 40 subjects. The two patients with detectable cell loss also showed defects in the specular reflex, the significance of which is discussed.

Key words: cornea - endothelium - non-contact - specular microscope - uveitis

Inflammation of the uveal tract is well known to have concomitant effects on the cornea. In acute anterior uveitis the corneal thickness increases (Matsushima & Polak 1969; Capella & Waltman 1971) and following the acute attack changes in the specular reflex of the endothelium have been described (Vogt 1930).

The increase in corneal thickness associated with the acute inflammation suggests that the barrier function of the endothelium is temporarily disturbed and raises the question of whether this is due to a loss of cells.

Due to the limited proliferative power of the adult human endothelium (Matsushima et al. 1966; Stocker 1971) and the high concordance between cell counts from right and left eyes in normal eyes (Sturrock et al. 1978; Olsen 1979, 1980).

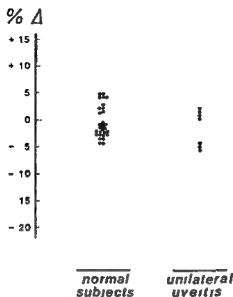


Fig 1

the inter-eye difference in cell counts in 13 patients with previous unilateral anterior uveitis (ordinate = $\frac{\text{affected eye} - \text{unaffected eye}}{\text{unaffected eye}} \times 100\%$) as compared to the relative difference in 36 normal subjects (abscissa = $\frac{\% \text{ eye} - \text{contralateral eye}}{\text{contralateral eye}} \times 100\%$ % denotes randomly chosen right or left eye).

they were seen as old large sharply demarcated precipitates. Besides the occasional occurrence of scattered physiological guttae none of the other patients showed this type of change in the specular reflex.

Central corneal thickness (CCT) was measured in both eyes of 47 normal subjects. 95% confidence limits for the normal difference between thickness of the right and left eye (CCT left eye - CCT right eye) was -0.013 mm and +0.016 mm. In practical terms this means that a difference of 0.02 mm or more is abnormal. From Table 1 it is seen that three of the patients (Nos 5, 8 and 10) had a CCT on the affected side which differed more than normal from the contralateral side. These patients also showed a faint aqueous flare on the affected side. In two of these patients a detectable cell loss had been found.

patients with detectable cell loss in the present study also showed numerous defects in the endothelial reflex. One of these patients also had elevated intraocular pressure so that an effect of this on the appearance of the endothelium cannot be excluded (Setälä & Vannas 1978). The other patient did not show this complication. The observed defects were clearly different from ordinary guttae. Vogt (1930) described excrescences on the posterior aspect of cornea after inflammation as irregular rather than ordinary guttae. The defects in the endothelial reflex seen in the present study were however large and almost circular giving an irregular outline only when they were confluent. In contrast to senile physiological guttae the cellular outline of the endothelium lining the posterior aspect of these excrescences could not readily be seen by altering the angle of illumination or viewing depth. The impression was that of a hyaline material deposited between the membrane of Descemet and the endothelial layer. In experimental uveitis inflammatory cells have been shown to enter this subendothelial space (Inomata & Saito 1970). It is tempting to speculate that if the defects in the specular reflex seen in the present study constitute remnants of precipitates formed in the acute phase of the inflammation they have gained access to the subendothelial space through a breakdown of the endothelial layer. Persisting precipitates seen as defects in the specular reflex may thus be indicative of endothelial cell loss. In the two patients showing a decrease in the cell density on the affected side also a thicker cornea on that side together with a faint aqueous flare. It is difficult to ascribe this minor increase in thickness to a relative decompensation of the endothelium as a result of damage in the acute phase or due to the still present inflammation in the eye. The one patient showing an increase in thickness without a decrease in cell density suggests the latter possibility.

Acknowledgments

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Fig 2

in appearance of two corneal endothelia which showed
 following acute anterior uveitis. Top: guttae-like lesions
 1 year-old male with recurrent attacks of iridocyclitis. Bottom:
 lesions in the endothelial reflex of 35-year-old female with
 attacks of panuveitis. Bar = 100 μ m

Discussion

Since Leber's time (1879) a destruction of the endothelium has been associated with abnormal hydration of the cornea. The specular microscope enables the degree of abnormal hydration to be quantitated if it has implied a loss of endothelial cells.

The patients included in this study have all had increased thickness of the cornea during the acute phase of their anterior uveitis. Of the 15 patients (15%) showed a significant cell loss on the affected side. In the patients at least part of the abnormal hydration of the cornea noted during the acute phase of the inflammation can thus be ascribed to a destruction of the endothelial cell layer. In the great majority of the patients however, no destruction could be detected. Therefore in these patients other factors must be sought for the increased hydration of the cornea. Whatever the exact factors may be the possibility remains that they may act by increasing the permeability of the endothelium without causing actual cell death.

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THE ENDOTHELIAL CELL DAMAGE IN ACUTE GLAUCOMA ON THE CORNEAL THICKNESS RESPONSE TO INTRAOCULAR PRESSURE

BY

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In order to assess a possible damage to the corneal endothelium during pressure induced abnormal hydration of the cornea 25 patients with a previous attack of unilateral acute glaucoma were photographed with the specular

microscope. As compared to the healthy side the endothelium of the affected eye showed a mean decrease in cell density of 23 % (range -4.8 to 68.7 %).

In retrospect this cell loss was found to correlate significantly to the change in corneal thickness measured during the acute attack on first day of

At present examination mean central corneal thickness was identical in affected and unaffected eye. A large variation was, however, found in intraocular pressure of the previously attacked eye. If the subject inter-eye difference in intraocular pressure was related to the inter-eye difference in corneal thickness a significant negative correlation appeared.

It is concluded that the intraocular pressure has a dual effect on the corneal thickness: if the endothelium is intact the intraocular pressure decreases corneal thickness, whereas an increase is seen only if the endothelium is acutely

cornea - endothelium - acute glaucoma - corneal thickness - intraocular pressure - specular microscope

Swelling of the cornea may occur as epithelial and/or stromal oedema, a well known clinical feature of increased intraocular pressure. It is often explained to occur when the interstitial fluid pressure of the cornea is positive (Ytteborg & Dohlman 1965a) and may as such occur

independently of intraocular pressure on the total corneal hydration, of which

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the shortest time period between onset of attack and present examination was one month to 13 months. One patient with haemorrhagic glaucoma was receiving antiglaucomatous treatment on the affected eye while the rest of the patients either received the same medical treatment to both eyes (three patients) or were without medication at all. The control group comprised 39 subjects in the age range 50-97 years (mean 72 years) with no history of eye disease or trauma other than senile lens opacities. Some of these subjects have been included in the normal series previously reported by Olsen (1979). The central endothelium of both eyes of patients and normal subjects were photographed and central endothelial cell densities estimated as described earlier (Olsen 1979). From the control group a normal relative inter-eye variation in cell density was constructed by randomly choosing right or left eye as reference for the observed difference in cell counts. By this method the small systematic right-left difference observed in normal eyes (Olsen 1979) is corrected. In the acute glaucoma group the difference in cell counts observed between the affected and the unaffected eye was expressed as relative to the unaffected eye. In order to ascertain a possible damage to the endothelium during glaucoma-operation 7 eyes were photographed before and after (range 3-5 months) a trabeculectomy or iridectomy in that eye. Central corneal thickness was measured using a modified Haag Streiff pachometer (Ehlers and Jørgensen 1977). Intraocular pressure was measured using a Goldmann applanation tonometer attached to the slit lamp.

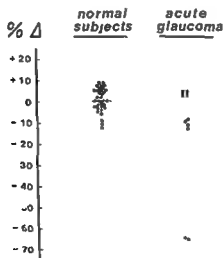


Fig. 1

inter-eye differences in cell counts in 93 patients with previous attack of acute glaucoma on one side

relative difference = $\frac{\text{affected eye} - \text{unaffected eye}}{\text{unaffected eye}} \times 100\%$ as compared to the relative inter-eye difference in a group of normal subjects

relative difference = $\frac{\text{x eye} - \text{contralateral eye}}{\text{contralateral eye}} \times 100\%$ x denotes randomly chosen right or left eye

stromal hydration constitutes a large part is however a confusing and controversial subject in ophthalmological literature. Does the intraocular pressure oppose the swelling of the stroma or does it oppose the swelling tendency of the cornea?

The clinical index of total corneal hydration is the corneal thickness (Watt 1968). In clinical studies both increased (Nyteborg & Dohlman 1963; Petráš et al. 1976) and decreased (Sbordone 1953; Olson & Kaufman 1964) corneal thickness have been observed with increased intraocular pressure. An increase in corneal thickness following a decrease in intraocular pressure has also been observed (Ehlers & Ruse 1967; Ehlers 1970). In vitro experiments have shown the corneal thickness to increase (Harris et al. 1956; Maurice & Hoesle 1963) and decrease (Maves & Hodson 1978) corneal hydration in experiments where the endothelium is claimed to be intact.

The above mentioned studies have largely dealt with intraocular pressures within the physiological range and elevated pressures as they are encountered in primary angle and secondary glaucomas. In severe cases of increased intraocular pressure, e.g. in acute glaucoma, there is general agreement that, in addition to the corneal oedema, a stromal oedema may develop (see Fuenne 1969; Charlier 1965).

Since Leuker's time (1873) the corneal endothelium has been known to be a factor for the water permeability of the cornea. A study on the state of the endothelium during pressure induced abnormal hydration of the cornea is deemed indicated.

Subjects and Methods

Twenty three patients in the age range 50-81 years with a mean of 69 years participated in the study. They comprised all patients who had been admitted to the eye clinic within two years with one unilateral attack of acute glaucoma. Nineteen patients (15 were men) had suffered from acute angle closure glaucoma, while 4 patients (3 were men) had acute haemorrhagic glaucoma. Patients with any other history of disease of either eye with the exception of senile lens opacities were excluded. Only patients who showed corneal oedema with epithelial bullae as indicated in the case records participated. In all patients the actual corneal thickness had been measured on the first day of admission by the doctor in charge or in some cases by the author. Following the acute attack, all patients were trabeculectomized or iridectomized on the affected side. In the angle closure patients were a few months later iridectomized on the other side, while the haemorrhagic patients were unoperated on the other eye at time of the present examination. In all patients where iridectomy was performed on the other eye only three days prior to the examination, the pachymetry readings were discarded in these patients while estimates were not. In the remainder the postoperative period for the control eye did not exceed more than two months.

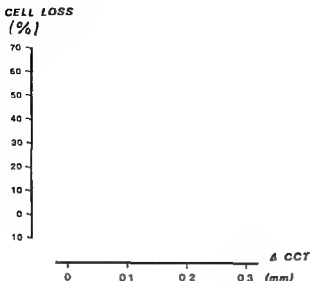


Fig 2

increase in central corneal thickness (CCT) on the first day of admission related to the related cell loss on the affected eye (Spearman's rank correlation coefficient $r_s = 0.68$, $P < 0.05$, $n = 11$)

day of admission the increase in corneal thickness on the affected side as compared to contralateral side was found to be significantly correlated to the cell loss (Fig 2). Attempts at correlating the cell loss to the visual acuity after the acute attack were unsuccessful.

In the present follow up the corneal thicknesses on the affected side were not different from the unaffected side. Mean central corneal thickness (\pm SD) was 11.515 ± 0.021 and 11.516 ± 0.026 mm respectively. The corneal thickness showed no trend towards a gradual change versus time in the post attack period studied. None showed epithelial oedema. Mean intraocular pressure (\pm SD) was 11.2 ± 8.8 (range 2) and 16.5 ± 4.2 (range 12-26) mmHg on the affected and unaffected side respectively. As can be seen the main difference existed in the much larger variation in intraocular pressures found in the eye with previous attack of acute glaucoma. The inter-eye differences in intraocular pressure were plotted against the inter-eye differences in central corneal thickness; a significant negative correlation appeared (Fig 3). Thus, in this situation where the patients served as their own controls the intraocular pressure was found to decrease the corneal thickness.

Results

On the average the cell count on the side with previous attack of acute glaucoma was 23.1% lower than the contralateral side with a large range in the difference (Fig. 1). In the normal group the inter-eye difference was 1.2% with a standard error of 5.7%. This means that 95% of the normal inter-eye difference was less than 11.4% following the procedure of randomly choosing left or right eye as reference. In this way 13 of the patients could be said to have significant endothelial cell density on the affected side. The counts themselves however do not show any definite grouping into normal and abnormal differences if taking the group as a whole the inter-eye differences were highly significant, different from zero. (A Wilcoxon test for paired differences showed $P < 0.001$).

The subgroup of acute haemorrhagic glaucomas showed a mean decrease in cell density of 16.4% (range 4 to 46%). In either group the cell loss was measured some time after the attack.

Table 1 shows the cell counts obtained in 7 patients undergoing a glaucoma operation. Mean change in cell counts were found to be -1.3% with a standard error of independent cell counts obtained with the present method. This has previously been shown to be 3.7% (Olsen 1979). Using this figure and assuming a normal distribution the 95% confidence limits for the observed mean value were calculated to be -2.7 and +0.1% respectively. Thus no significant change occurred during the operation and if there was any it was very small. This indicates the cell loss depicted in Fig. 1 not to be iatrogenic but to be a result of the attack.

In those instances where corneal thickness measurements were available

Table 1
Estimated endothelial cell density in 7 patients before and 3-5 months after glaucoma operation. Mean change in cell density is -1.3%

Patient No	Operation	Cell count before (cells/mm ²)	After (cells/mm ²)	%
1	iridectomy	1430	1370	95.8
2	trabeculectomy	2379	2340	98.4
3	iridectomy	2822	2780	98.5
4	trabeculectomy	1897	1863	98.2
5	trabeculectomy	9003	8840	98.2
6	trabeculectomy	2579	2510	97.3
7	trabeculectomy	3039	2970	97.7

fore strongly indicative of the irreversible cell loss having occurred during the attack of glaucoma. The results of Svedberg (1975) suggest that the pressure may destroy the endothelial layer but it may be suggested that additional factors such as speed of onset, ischemia and anaerobic metabolism due to impaired outflow of aqueous may also contribute to the endothelial damage.

The endothelial damage had important consequences on the corneal hydration as shown in Fig. 2. It was not tried to correlate the duration of the attack and the actual intraocular pressure to the cell loss due to difficulty in precise determinations of these variables. It may be suggested, however, that the cellular destruction was the final common path of these variables to increase the corneal hydration.

Watt (1956) noted an association between a low number of endothelial cells and a tendency of the cornea to become oedematous at increased intraocular pressure. He concluded, however, the low cell density as such to be the cause of the abnormal hydration. The results of the present study indicate that it is the active shedding of cells that matters. It is remarkable that even if more than 50% of the endothelium had been destroyed it did not seem to have a permanent effect on corneal hydration. Mean corneal thickness of the previously attacked eye was found not to differ from the unaffected eye. The latter result is in accordance with other authors (Watt 1956, De Cevallos et al. 1976).

In the present follow up examination the patients turned out to have a large variation in the intraocular pressure in the previously attacked eye. Using the contralateral eye as control the intraocular pressure was shown to be inversely correlated to the corneal thickness (Fig. 3). In interpreting these results it is important to remember that the patients at this time had an intact endothelium as evidenced by the intact endothelial reflex (Fig. 4). During the acute attack with increased corneal thickness it has never in hands of the author been possible to observe a normal endothelial reflex (Fig. 5).

The results of the present study therefore indicate that the intraocular pressure increases corneal thickness if the endothelial layer is damaged but decreases the corneal thickness if the endothelial layer is preserved. The hypothesis that the state of the endothelium may switch the thickness response of the cornea in either direction has been suspected by some authors (Mishima 1968, Ehlers 1970) but not documented.

In animal experiments Ytteborg & Dohlman (1965) suggested that the increased intraocular pressure might have damaged the endothelium for the increased corneal thickness to occur. The clinical data reported by these authors (Ytteborg & Dohlman 1965a) are however inconclusive on that point since many of their patients must have had secondary glaucoma where damage to the endothelium via other routes (operation, inflammation) cannot be ruled out (Olson & Kaufman 1978).



Fig. 4

The en^d helial reflex in a patient with a previous attack of unilateral optic
 atrophy. Tip: Endothelium of affected eye. Faint red orⁿ down
 cells. Bottom: Endothelium of normal fellow eye. Faint red orⁿ down
 cells. Bar indicates 100 μ m.

the fact that the cornea with intact endothelium thins in response to elevated ocular pressure raises some theoretical questions as to the function of the endothelium in this situation. If the endothelium was highly impermeable to water it would be understandable that an increased pressure gradient across the cornea would make it thin. The endothelium has however failed to demonstrate such an impermeability to water in the rabbit (Mishima & Hedbys 1967) and in the human (Nelson 1972). The fact that the cornea does not swell *in vivo* has therefore called into question the existence of an active endothelial pump which constantly pumps fluid out of the stroma into the aqueous (Mishima 1968; Maurice 1969, 1972; Hodson & Miller 1973). On basis of this model a very leaky endothelium and a large pumping capacity the hydration of the cornea would be expected to increase if the pressure gradient across the endothelium was raised.

In view of the present findings and other reports showing an inverse relationship between IOP and corneal thickness at presumed intact endothelium (Ehlers & Riise 1970; Ehlers 1970; Olson & Kaufman 1978) it seems that if the pump-leak concept of the function of the endothelium is to be maintained it would require the pump to be activated or the leakage to be lowered in response to an increased intraocular pressure as long as the pressure does not damage the endothelium.

Acknowledgments

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ENDOTHELIAL CELL DENSITIES IN DONOR AND RECIPIENT TISSUE AFTER KERATOPLASTY

BY

PEKKA RUUSUVAARA

A specular microscope was used to photograph the cell densities of the central cornea of the graft and the peripheral recipient cornea in a total of 32 human eyes. The follow up period varied from 8 months to 13 years with an average of 4.7 years.

The mean endothelial cell count in the graft was 997 ± 356 cells/mm² and 1410 ± 584 cells/mm² in the recipient's peripheral cornea. The mean difference in cell counts between donor and recipient tissues was 413 ± 718 cells/mm² (99%).

There were great disparities between graft and recipient cell densities. One patient with keratoconus had a graft/recipient differential in cell density of 3 to 1. In four cases the cell density was higher in the graft than in the recipient. Of these grafts two had been stored in M.H. medium and two had been cryopreserved. The great difference in cell density observed between the graft and recipient corneas strongly suggests that little if any cell migration occurs between graft and recipient. This suggests that the scar tissue forms a barrier to cell migration between graft and recipient.

Key words: endothelium - cell density - cell migration - corneal graft - corneal preservation - contact specular microscopy

Simultaneous division of corneal endothelial cells has not been demonstrated to occur in man. In a study of eye bank eyes a rare mitosis was observed in endothelium stained with para-nitro-blue tetrazolium (Kaufman et al. 1966). Dead human endothelium is believed to heal by spreading and sliding of the remaining live cells to cover the areas of dead cells and not by cell division. This

presumably explains the larger corneal endothelial cells and lower cell counts observed after certain operative procedures.

In 1968 Maurice developed a specular microscope for studying the endothelium. Laing et al (1975) and Bourne & Kaufman (1976a) described this instrument for clinical use and since then much information has been gained about the behaviour of the human corneal endothelium.

In their specular microscope studies Blackwell et al (1977) and Srinivasan (1978) showed that the human cornea has similar cell counts at the centre and periphery and that pairs of eyes have similar axial cell counts. Likewise, studies of normal eyes. Laule et al (1978) found that in most cases counts of the endothelial cell population of one eye are closely correlated with the corresponding counts of the fellow eye.

In view of the restricted capacity of the human endothelium to divide and the cell densities and cell morphology in corneas to be used for human grafts are very valuable. A question of the utmost importance is the origin of the endothelial cells that are present in a human corneal graft after long term follow-up. A speculation exists about the capacity of human endothelial cells to migrate from the graft to the recipient or conversely from the recipient to the graft (Brown 1971; Bourne & Kaufman 1976c; Polack 1977).

The purpose of this paper is to compare the endothelial cell densities of corneal grafts with those of the peripheral recipient cornea. A conclusion is drawn about the question of whether human endothelial cell migration occurs between donor and recipient tissue sites.

Patients and Methods

I have previously presented a clinical series comprising 102 penetrating keratoplasties in 90 patients (Ruusuvaara 1979 a, b). The patients of the present study are a subgroup of that series comprising 32 penetrating keratoplasties in 26 patients. These grafts were bilateral. The follow-up period varied from 8 months to 12 years with an average of 4.7 years. The ages of the patients ranged from 2 to 78 years and those of the donors from 13 to 78 years. The ratio of males to females was 3 to 1.

There were altogether 31 eyes with keratoconus and one patient had dystrophy. A contact specular microscope (Seyber Inc.) was used to photograph the central cornea of the graft and the recipient cells at the periphery. In 14 cases the peripheral endothelium of the unoperated fellow eye was also photographed. Photographs of the cornea at the centre and periphery of a fixation

fixed to the wall. This enabled the patients to maintain the appropriate gaze. Eye position was also checked by intermittent direct observation of the eye. The lateral recipient cornea was photographed in the nasal and temporal areas as possible. For each eye 10 to 20 photographs were taken at the periphery and centre of the cornea and 5 of the clearest from each area were selected for printing and counting. The prints had a final magnification of $\times 500$.

Operative methods

Operations were mostly performed by the same two surgeons. The size of the graft was 7 mm in 20 cases and 8 mm in the rest. Operative methods and instruments used are described in an earlier paper (Ruusuvaara 1979a).

Methods of corneal preservation

Patients were grouped according to the method of preservation of the donor corneas. One group comprised 11 patients with grafts which had been stored in a moist chamber as whole globes at 4°C for less than 48 h. Donor corneas were transplanted after an intermediate term of preservation (days) in the M K medium (McCarey & Kaufman 1974). Cryopreservation (Kaufman & Capella 1968) had been used for 16 donor corneas with a preservation time from 8 days to 4 years.

Results

In all 39 patients the mean endothelial cell count in the graft was 997 ± 306 cells/mm² (Table 1). The mean endothelial cell density in the recipient periphery was 410 ± 584 cells/mm². The mean difference in endothelial cell counts between graft and recipient tissues was 413 ± 718 cells/mm² (29%). In the 8 patients in whom the periphery of the fellow eye was also photographed the endothelial cell density in the periphery of the operated recipient eye was 1742 ± 688 cells/mm², i.e. that of the fellow eye 2918 ± 310 cells/mm², the unoperated eye having 68% as many cells at the periphery than the operated eye.

Further analysis of cell densities in graft endothelia showed that grafts had significantly more cells after storage in M K medium than after cryopreservation or storage in a moist chamber. Moreover, of the two latter groups the cryopreserved grafts had significantly more endothelial cells ($P < 0.001$). The follow up period was longest (8½ years) for the corneas grafted after moist chamber storage and shortest (2¼ years) for cryopreserved corneas and corneas stored in M K medium.

Patient	Rever- sion method	Donor age (years)	Culture time	Prever- sion time	Follow up period	Mean cell density of grafts (cells/mm ²)	Mean cell density of recipient peripheral embryal (cells/mm ²)	% of difference	Fellow eye cell count (cells/mm ²)
1	crn	47	6h	1 mo	3 yrs 7 mo	663	1217	21	3067
2	-	47	0	1 mo 29 d	1 yrs 5 mo	858	1275	33	-
3	-	43	2h	4 mo 25 d	4 yrs 2 mo	812	1133	11	-
4	-	72	0	8 mo 1 d	2 yrs 2 mo	98	2508	62	3202
5	-	66	0	2 yrs 11 mo	9 mo	1133	1400	19	-
6	-	43	9h	3 mo 21 d	3 yrs 11 mo	1525	725	-13	-
7	-	70	h	11 mo 29 d	1 yrs 9 mo	108	1198	7	-
8	-	73	3h	2 yrs 3 mo	11 mo	1070	210	6	-
9	-	79	2h	11 d	3 yrs 6 mo	195	1133	18	-
10	-	72	0	3 yrs 11 mo	10 mo	1533	1704	1	-
11	-	55	0	1 mo 18 d	8 mo	1138	118	2	-
12	-	6	0	3 yrs 2 mo	1	1000	0.5	-100	-
13	-	4	0.1	4 mo 16 d	3 yrs 11 mo	1070	100	41	-
14	-	-	2h	3 mo	3 yrs 4 mo	100	100	0	-
15	-	-	2h	3 mo	3 yrs 4 mo	100	100	0	-

n	st chamber	mm	15 h	9 h	8 yrs 4 mo	C50	124	19	-91
17	-	11	15 h	9 h	8 yrs 4 mo	C50	124	19	-91
18	-	4	15 h	1 h	8 yrs 4 mo	809	1093	22	-
19	-	95	11 h	1 h	6 yrs 5 mo	175	909	26	-
20	-	19	5 h	21 h	6 yrs 6 mo	50	2702	80	2912
21	-	26	15 h	1 h	8 yrs 5 mo	975	1258	90	-
22	-	-	0	1 h	1 yrs 11 mo	959	1077	24	-
23	-	54	1 h	9 h	6 yrs 11 mo	963	899	2	-
24	-	98	5 h	24 h	5 yrs 11 mo	712	1233	10	2858
25	-	62	9 h	-	10 yrs 9 mo	612	917	21	-
26	-	69	9 h	49 h	8 yrs	917	992	8	-
27	-	10	12 h	9 h	12 yrs	757	1113	34	-
Total									
(most chamber)									
11	94	8 h	11 h	8 yrs 3 mo	709 ± 120*	1231 ± 44*	9876	-	-
M h median									
28	-	95	9 h	4 h	9 yrs 6 mo	1010	1299	19	-
29	-	99	5 h	4 d	9 yrs 5 mo	772	1292	40	2999
30	-	91	0	3 d	9 yrs 6 mo	2109	750	-191	-
31	-	99	9 h	9 d	2 yrs 5 mo	910	9993	60	9159
32	-	39	1 h	9 h	1 yrs 7 mo	9172	1906	-72	9210
Total (M h)									
35	35	9 h	3 d	2 yrs 3 mo	1409 ± 66*	190 ± 99*	±0	-	-
All cases									
37	33	1 1/2 h	-	47	997 ± 956*	1410 ± 991*	997	9918 ± 310*	-

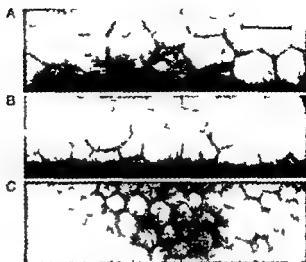


Fig 1A B C (patient no 17)

Specular micrographs of corneal endothelium. A Graft (preserved by moist chamber for 3 h) B peripheral endothelium of recipient C peripheral endothelium of fellow eye. Cell densities of graft recipient and fellow eye are 650 mm^{-2} 1040 mm^{-2} and 900 mm^{-2} respectively. Scale bar 50μ



Fig 2A B (patient no 30)

A Central corneal endothelium of a graft stored in M K medium for 3 days B endothelium of recipient. Cell densities of graft and recipient are 9100 mm^{-2} and 950 mm^{-2} respectively. Scale bar 50μ



Fig 3A B (patient no 12)

l corneal endothelium of a graft cryopreserved for 1 year and 2 months
ral endothelium of recipient with clearly dystrophic endothelial cells (Fuchs) Cell
nsities of graft and recipient are 1000/mm² and 600/mm² (x 200 bar 50 μ)

dothelial cell densities at the periphery of the recipient corneas did not
ificantly from each other in the different preservation groups although
ents of grafts stored in a moist chamber had 300 fewer cells/mm² than the
of cryopreserved grafts ($P > 0.05$). Thus it seems that the differences in
lation density between the graft and the peripheral recipient cornea are
it mainly on the cell density of the corneal graft itself the difference
graft and recipient being greatest in the group that had received grafts
a moist chamber. In this group the difference in cell density between graft
nent was 466 ± 613 cells/mm² which means that the peripheral recipient
ad 72% more endothelial cells than the graft. In 5 grafts stored in M K
there was no difference in average cell densities between graft and
In the 16 cryopreserved corneal grafts the cell density in the recipient

al cornea was 55% higher than in the graft
and recipient cell densities showed great disparities (Fig 1A B C). One
ith keratoconus (patient No. 30) had a graft/recipient differential of 3 to 1
B).

igh in most cases the cell density was higher in the recipient tissue in four
as higher in the graft. Two of these grafts had been stored in M K medium
l 3 days (Fig 2A B) two had been cryopreserved. One of the latter was in
nt with Fuchs' dystrophy (patient No. 12) (Fig 3A B). No dystrophic cells
ind in the graft whereas nearly all the peripheral endothelial cells were
dystrophic with non reflecting areas.

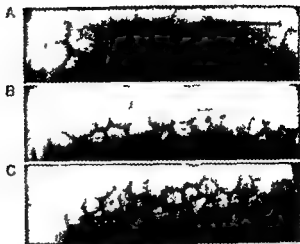


Fig 4A, B, C (patient no. 20)

A Central corneal endothelium of a graft preserved by moist chamber 4) B B recipient endothelium and C peripheral endothelium of fellow eye. Cell densities of recipient and fellow eye are 550 mm^2 , $2792/\text{mm}^2$ and $2941/\text{mm}^2$ (A, B, C).

The maximal difference in cell density between graft and recipient was for patient with keratoconus (patient No. 20). This donor graft had been in moist chamber and there had been an episode of graft rejection 3 years after operation. The graft cell density was 550 cells/mm^2 and the recipient's 2792 cells/mm^2 , a difference of 1 to 5 (Fig 4A, B, C).

Table I shows the mean donor age, cadaver time, preservation time, period, mean cell densities of grafts and recipients in each individual of the different preservation groups.

Discussion

In human studies there has been speculation but few actual data are available about the migration or spread of endothelial cells from the recipient to the graft or in the opposite direction (Bourne & Kaufman 1976c). Brown & Brown (1971) believed that a given percentage of non-viable cells in the donor cornea must be removed by the sliding of cells from the recipient. They also believed that in Fuchs' dystrophy, where the viability of the recipient cells is already questionable, cells may migrate from graft to recipient, especially with larger grafts. In a study with an 11-month-old clear penetrating corneal transplant, Bourne & Brown (1976b) observed a significant increase in endothelial cell density after

They believed that the increased number of central endothelial cells must arise either by cell division or by sliding of cells from the periphery of the graft or from the recipient tissue.

Cellular microscopy after keratoplasty shows a uniform cell density within the clear graft portion of the endothelium (Liang et al. 1976). The cell density in the recipient portion is likewise uniform. Yet it is reasonable to assume that at the time of surgery cell loss will vary from one area to another. It will probably be greatest at the centre of the graft, which may be pressed against the iris and lens especially when the anterior chamber is shallow and along the peripheries of both graft and recipient tissues where suturing would be accompanied by localized trauma.

Animal studies have shown that rabbit donor endothelium can repopulate the recipient cornea when there is a deficiency of recipient endothelial cells. This was shown by Olson & Levenson (1977) using sex chromatin as cell marker. Bourne (1978) in his study with monkeys concluded that more cells of the recipient type are usually found in the periphery than in the central part of the graft. He took this evidence of cellular migration from host to graft. In other studies (Gospodarowicz 1979) no invasion of the corneal button by the recipient endothelial cells and likewise no invasion of the recipient endothelium by the donor cells could be demonstrated in bovine or rabbit transplants.

In my study comparison of the cell densities in the corneal graft and the peripheral recipient cornea showed very great disparities. In most cases the cell density was found to be much lower in the graft than in the recipient. The difference was highest ($550 \cdot 2 \sim 92$ cells mm^{-2}) in a patient with keratoconus who had a rejection episode in the transplant 2 years before photography. It seems reasonable to assume that if in his case any cells had migrated from the periphery of the recipient to the graft the differences in cell densities would not have been so great (a differential of 1:5). Also in the patient with Fuchs' dystrophy all the graft cells were hexagonal and normal-looking and all the peripheral recipient cells typically dystrophic. So no normal cells seemed to have migrated from the transplant to the dystrophic recipient area.

In eight cases it was possible to compare the peripheral endothelia of the transplanted eye and its fellow. The mean difference in cell density between the transplanted and unoperated eye was more than 1000 cells mm^{-2} . Thus it seems that in keratoplasty we lose an average of 41% of cells from the recipient peripheral endothelium. Thus in performing keratoplasty one must remember that the recipient corneal endothelium is also vulnerable and may suffer later complications from operative damage.

According to this study it seems that little if any cell migration occurs between donor and recipient tissues. Supporting this view is the observation of great disparity

between population densities in both directions across the donor-recipient interface. In some cases it was the graft and not the recipient cornea that had the higher density. In those cases in which the endothelial cell densities of graft and recipient were virtually the same we can speculate that cells were also lost from the peripheral cornea during the surgical manipulation, especially the suturing. Although it seems that endothelial cells can slide with the graft and within that of the peripheral recipient cornea to cover the interface, this study strongly suggests that no cell migration occurs across the interface between graft and recipient.

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ENDOTHELIAL CELL DENSITY IN DONOR CORNEA

BY

STEFFEN SPERLING

In 64 pairs of donor corneas the endothelial cells were visualized by provoked swelling of the cell borders in isotonic saline. In corneas from donors of various ages numerical cell density was correlated to age. This was not done in corneas from older donors. The cell densities in paired corneas were correlated. The cell density in the second cornea of a pair was found to within $\pm 10.97\%$ ($\pm 1.99\%$) of the first cornea. The precision of this result could only be improved insignificantly by correction for age, sex, corneal density or variation of the mean.

Key words: human cornea - endothelial density - contralateral cornea - unbiased counting

If the demand for donor corneas with high endothelial cell density exceeds the supply from young donors, available tissue from older donors can be used in individual evaluation. If a direct view of the endothelium has been obscured by corneal oedema, or if a specular microscope is not available, the endothelial density can be determined in an excised cornea by an ordinary light microscope after provoked swelling of the endothelial cell borders in isotonic saline (Sperling & Larsen 1979).

This study was undertaken in order to evaluate the precision of an estimate of endothelial cell density in a donor cornea based on observations in the contralateral cornea, and in order to evaluate whether knowledge of donor age or sex is relevant to the precision of the estimate.

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Material and Methods

hundred and thirty six cadaver corneas from 34 patients of each sex aged from 15 to 92 were included in the present study. Whole eyes were enucleated when no history of eye disease appeared from the case record and when the conjunctiva, the cornea and the anterior chamber appeared normal by simple inspection. Enucleated eyes were discarded when signs of inflammation, trauma or surgery were found by slit lamp examination. Corneas were prepared with a scleral rim and the endothelial cell borders were visualized by osmotic swelling in isotonic saline (Sperling & Larsen 1979). Microphotographs were taken at a total linear magnification of 162x on Polaroid type 667 8.3 x 10.8 cm. The microscope was set at random within the central 6 mm of cornea and three photographs were taken when two thirds or more of a full field could be brought in focus. Two photographs were selected on basis of the largest photographic clarity. On each photograph cells were counted in two test frames of 165 x 325 mm. The procedure of unbiased counting described by Sperling & Gundersen (1978) was applied.

Results

For each cornea the sex, the mean endothelial cell density (\bar{x}), the standard deviation ($SD(\bar{x})$) and the coefficient of variation ($CV(\bar{x}) = \frac{SD(\bar{x})}{\bar{x}} \cdot 100$) was registered.

For each pair of corneas the relative difference between contralateral mean cell densities ($Dr = \frac{|\bar{x}_1 - \bar{x}_2|}{\bar{x}_1} \cdot 100$) was determined. One cornea of each pair was chosen at

random as cornea 1 without regard to original right-left situation. The mean values and some correlations between the registered parameters are indicated in table 1. As no difference was found between male and female corneas the coefficients of correlation are also indicated for the total material comprising 68 pairs. In 68 paired values correlation is significant ($2P < 0.05$) when $r > |0.245|$ (Snedecor & Geigy 1970).

A highly significant correlation was found between cell densities in paired corneas ($r = +0.94$, $n = 68$, $2P < 0.001$). In the total material the coefficient of correlation between age and cell density (r_1) was -0.27 ($0.025 < 2P < 0.05$, $n = 68$). Cell density was correlated negatively to age in 18 corneas from 11 donors of less than 50 years of age ($r = -0.62$, $n = 18$, $0.005 < 2P < 0.01$) while this was not the case in 118 corneas from 59 older donors ($r = +0.06$, $n = 118$). A negative correlation was also found between the cell densities and the relative differences between mean densities of paired corneas ($r = -0.30$, $n = 68$, $0.025 < 2P < 0.05$). The correlations between the relative difference and age and between the relative difference and coefficient of variation ($CV(\bar{x})$) were just below significant values ($r = 0.23$ and $n = 0.22$ respectively, $2P > 0.05$).

Table I

Correlations between mean cell density (\bar{x}) coefficient of variation on the mean ($CV(\bar{x})$) relative difference between mean cell densities in contralateral corneas (D) and age are

x_1	Sex	\bar{x}	$r_{xy} (n = 31)$	$r_{xy} (n = 62)$
$x_1 \times$	M	2992.21 2997.38	0.91	0.91
	F	2726.00 2729.97	0.93	
$D \times_1$	M	5.99 2992.21	-0.18	-0.30
	F	4.53 2629.00	-0.09	
$D \times_{age}$	M	4.00 65.75	0.1	0.23
	F	4.53 61.00	0.29	
$D \times CV(\bar{x})$	M	1.00 4.59	0.50	0.2
	F	4.50 9.89	0.15	
$x_1 \times_{age}$	M	2992.21 65.76	-0.1	-0.08
	F	2629.00 61.00	-0.30	

The distribution of relative differences between contralateral mean cell densities is shown in Fig. 2. On probability paper the differences showed a close fit to a normal distribution. When the differences were separated into positive and negative values a mean value of -0.33 and a standard deviation of 1.09 was found. This indicates that the cell density in the second cornea of a pair was within the interval $\bar{x} \pm 1.097\sigma$ ($\pm 1.98SD$) with a probability of 0.97 (Yates 1937).

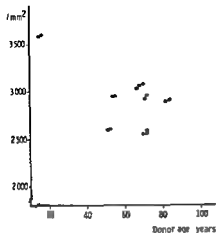


Fig 1

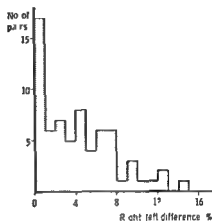


Fig 2

- 1 Central endothelial cell density related to age for one cornea from each of 68 pairs
- 2 Distribution of relative differences between contralateral endothelial mean cell densities $\left(\frac{|\bar{x}_1 - \bar{x}_2|}{\bar{x}_1} 100\% \right)$

Comments

■ corneas in the present study were selected by criteria applied for donor corneas. Corneas were obtained from the hospital morgue without regard to donor age. The overrepresentation of corneas from patients over 50 years of age (56%) reflects the high incidence of older patients dying in the hospital.

In the present material a strong positive correlation was found between endothelial cell densities in paired corneas. This indicates that the second cornea of a pair can be accepted or excluded for individual evaluation when the endothelial cell density is known in the first cornea. The tendencies towards decreasing correlation with increasing donor age, with low numerical cell density and with variation of the mean were weak. This indicates that the precision of an estimate of the endothelial density in the second cornea of a pair can only be improved slightly by correction for donor age, mean cell density or variation of the mean.

In the present material endothelial cell density was negatively correlated to donor age when the total material was considered. A strong correlation appeared in 18 corneas from young donors while no correlation ($r = +0.06$) was found in 118 corneas from donors of more than 50 years of age. This indicates that whenever

donor tissue with high endothelial density is wanted there is no reason to favourer of two donors over 50 years of age

Endothelial cell density have earlier been related to age by Irvine & Irvine (1953) Bourne & Kaufman (1976) Laing et al (1976) Laule et al (1978) Sperling & Sturrock et al (1978) Olsen (1979). In the data published by most of these negative correlations between age and endothelial cell density were found in the age group 0-50 years. In corneas from older individuals age and cell density were correlated in either material

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DIABETIC RETINOPATHY IN VISUAL DEPRIVATION

A case report

BY

LUTZA YANKO

A case of asymmetric retinal involvement in a diabetic patient who has long been exposed to visual deprivation on one eye is described. The question implied by this condition is if a relationship could exist between the disjunct exposure of each eye to different visual excitations and the outcome of the different retinal diabetic involvement. The possibility that the retina in a resting state might be less prone to develop diabetic retinopathy than usual and a possible functional hypothesis on the pathogenesis of this diabetic complication are questioned.

Keywords: diabetic retinopathy - asymmetry - visual deprivation

lower than expected prevalence of diabetic retinopathy has often been reported in patients in whom other ocular conditions were present. In some circumstances a negative association has been observed between increased intraocular pressure and the development of diabetic retinopathy particularly proliferative retinopathy. Some of the work relating to this association between ocular tension and retinopathy has been reviewed by Keen (1972) and by Shin et al (1977). Other different ocular conditions accompanying retinal sparing have also been described occasionally in diabetics.

Clinical observations of asymmetric retinal involvement particularly when associated with other significant intraocular differences might be of some value in investigating possible causal associations and the pathogenesis of diabetic retinopathy.

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ACUTE OCULAR HYPOTONY AS A MANIFESTATION OF MUMPS

A Case Report

BY

JON ERIK SLAGSVOLD

A 27 year-old woman with epidemic parotitis developed acute hypotony in her right eye. This complication is not mentioned in earlier reports concerning the disease. She also suffered from keratitis and iritis. The inflammatory reaction of the anterior eye subsided on topical medication, but the hypotony persisted for two weeks. Factors possibly influencing the low intraocular tension are discussed.

Keywords: epidemic parotitis — keratitis — iritis — ocular hypotony

Case Report

A 27 year-old housewife was admitted to the Eye Clinic with two days history of pain and increasing hazy vision in her right eye. There was no history of previous eye disease. She had been exposed to mumps through affected family members, i.e. two daughters and father, none of whom had eye problems. Five days before admission she had developed characteristic swelling of both parotid glands.

Eye examination disclosed normal physical findings of her left eye. The right eye showed moderate ciliary injection, oedematous corneal epithelium, opaque stroma and Descemet's membrane (Fig. 1). Cornea was about twice as thick as in the fellow eye (pachymetry). Slit lamp examination of the anterior chamber revealed a trace of flare and cells, some fine corneal precipitates. The pupil had a normal size and reacted briskly. Visual acuity was 6/24 corrected. The intraocular tension, measured with Goldmann applanation tonometry, was 10 mmHg (left eye 14 mmHg). The eye was treated with atropine and prednisolone acetate 0.5% five times daily.

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Fig 1

eye showing central opaque parenchyma and folds in Descemet's membrane



Fig 2

not looking downwards to show the impression of enophthalmos (ptosis) caused by the hypotony

Five days later the corneal oedema persisted but the vision had improved 6/12 r however was so soft that it gave the impression of enophthalmos (Fig 2) ie π_{μ} tonometry 4 mmHg (Schötz 14/5 5) Glycerol eye drops temporarily removed oedema whereafter gonioscopic and funduscopic investigations with the three-mirror (Goldmann) were performed. No pathological changes were observed in retina or iridocorneal angle.

During the following three weeks all symptoms subsided. The enophthalmos disappeared, the visual acuity and the intraocular pressure gradually normalized (5.6-14 mmHg). Descemet's folds remained for three and a half months. Further follow up for one year showed normal intraocular tension.

Mumps serology not analyzed in family members confirmed the clinical diagnosis. Increase from 80 to 160 in seven weeks (complement fixation test).

Discussion

The diagnosis of mumps was based upon medical history (family members) & examination (parotid swellings) virus serology and leaves no doubt in this case.

Several ocular manifestations in mumps are known i.e. dacryoadenitis, conjunctivitis, keratitis, uveitis, scleritis, optic neuritis (Stenstrom 1943, Blax 1953, 1954, 1956, Katavisto 1956, Riffenburgh 1961). Five cases of glaucoma have been described (Stenstrom 1943, Roussel 1946, Riffenburgh 1954, Pollard & Thorpe 1976). Hypotony as a complication to mumps has to my knowledge never been reported previously.

The patient suffered from corneal oedema, iritis and hypotony. Corneal oedema in parotitis is exceedingly rare (Fields 1947) but represents a characteristic clinical picture (Riffenburgh 1961) i.e. usually unilateral & developing keratitis profunda with complete clearing. The iritis is usually a serous inflammation probably self limited leaving no synechiae or other sequelae. The findings in our case fits well with these descriptions. The corneal oedema could neither be explained by the mild iritis nor the hypotony which persisted for many days after the cornea had cleared up.

As to the cause of the hypotony different pathogenic factors may be considered. Intraocular pressure is influenced by the central nervous system. In experimental glaucoma has been observed in cases of meningitis of other origin & in experimental induction caused by mump virus in this case leading to low pressure is unlikely i.e. the patient had no signs of meningeal inflammation.

Detachment of the retina or choroid may be associated with hypotony (Pollard 1954). With maximal dilatation of the pupil paying special attention to the periphery these conditions were ruled out.

Iridocyclitis is often accompanied by low intraocular pressure. The inflammation

in of the anterior chamber was mild and subsided rapidly. However the my may be explained by a more specific affection of the secretory function of ciliary body. On the other hand Chandler & Maumenee (1961) found fluid in the sclera and the ciliary body in eyes with mushy soft tension following conjunctivitis. These eyes however suffered from a heavy inflammation which was not the case in our patient.

Hypotony without any obvious explanation following another viral eye disease herpes zoster was reported by Juler (1928). He suggested that atrophy of the ciliary body might be responsible.

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Short Communication

IMPROVEMENT OF CONTRAST SENSITIVITY FROM TREATMENT FOR AMBLYOPIA

BY

GUNNAR LENNERSTRAND and BJÖRN L. LUNDH

Twenty four amblyopic children were treated with grating visual stimulation. In eight of them visual acuity did not change. However, four of these children showed improved contrast sensitivity.

Key words: contrast sensitivity — visuogram — amblyopia — CAM treatment.

We have studied contrast sensitivity changes during treatment for amblyopic children and correlated them to changes in visual acuity. Monocular contrast sensitivity of both the good and the amblyopic eye was established before and after amblyopia treatment in children aged 5½ to 13 years. The method was similar to that used by Derffeldt et al (1979) except that only one determination of contrast threshold was made at each spatial frequency. Amblyopia was treated with the method of grating visual stimulation described by Campbell et al (1977). The deprived eye was exposed to high contrast grating patterns of different spatial frequencies while the good eye was occluded. Treatment periods of 10 min were repeated twice a week. Between sessions the children continued to wear their best spectacle correction. If no improvement of visual acuity was found after 10 sessions the good eye was part time occluded, e.g. all the time the child was away from school. Most children showed some increase of visual acuity for far and

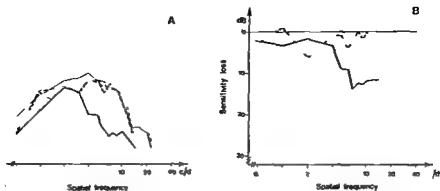


Fig 1

ges in contrast sensitivity from amblyopia treatment of the right eye of a ten year-old with anisohypermetropia and right microtropia. Snellen visual acuity was 0.2 of the right and 0.8 of the left eye before and after fifteen sessions of grating pattern stimulation.

Contrast sensitivity curves of both eyes before (full line) and after treatment (dashed line). Thicker lines denote the amblyopic right eye. The maximal contrast available was 43%.

Visuogram showing the left eye before (full line) and after treatment (dashed line). The contrast sensitivity at each spatial frequency has been related to values obtained in normal of children aged 6 to 10 years (see Derdikman et al. 1979). This sensitivity loss is expressed in dB. After amblyopia treatment the sensitivity loss is less marked in the 4 to 10 cycles per degree range.

In quite a few cases visual acuity remained at the same level even after fifteen sessions and parttime occlusion. In the group with improved visual acuity parallel changes were seen in the contrast sensitivity. More remarkable was the finding that changes of contrast sensitivity also occurred in children regarded as failures from standpoint of visual acuity only. An example is given in Fig. 1. This ten year old with anisometropia and microtropia had a Snellen acuity of 0.2 before and after treatment of his right amblyopic eye. However, after ten training sessions the contrast sensitivity of this eye showed a marked increase in the range of middle and high spatial frequencies (Fig. 1A). This is also illustrated with the visuogram of his amblyopic eye (Fig. 1B). Similar results have been obtained in four out of eight children who showed no change in visual acuity.

This improvement of contrast sensitivity from amblyopia treatment, without effect on visual acuity, has been clearly demonstrated in children. These results emphasize the importance of contrast sensitivity determination in the evaluation of amblyopia (Hess 1979) and in amblyopia treatment. Undoubtedly a normalisation of contrast sensitivity even for a restricted range of spatial frequencies represents a notable gain in the visual ability of an amblyopic eye.

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TRANSACTIONS OF
THE DANISH OPHTHALMOLOGICAL SOCIETY
1977-1978

BY

ERIK SCHERFIG Secretary

471st Meeting October 1 1977 in Copenhagen
(Rigshospitalet)

te Warburg *Blindness among 7742 mentally retarded children in Denmark.
o-medical aspects)*

We have performed a screening among 7742 mentally retarded children below 21 years of age and have examined 380 individuals suspected of being blind. Blindness was found in 82 of them. After the completion of the examinations, reports on blind children were still coming. The blind M. R. children observed represent 50% of all blind Danish children. Mildly retarded children make up at least 8% of the total group of normal, educationally normal and mildly retarded blind children in Denmark. The majority are severely or profoundly retarded (60%). Epilepsy was found in 70%, spastic palsy in 61% and hearing disorders in 14%. Of the children 37% could walk, 40% could sit in chairs while 19% could be down. Opac, atrophy, cortical blindness, tapeto-retinal degeneration and cataract are the most common diagnoses. retrolental fibroplasia was less frequent than among other children.

The principal causes of blindness were brain disorders; a genetic aetiology was disclosed in infections in 11% and prematurity without oxygen treatment in 6%. Blindness had not been recognized in one third of the group prior to this study. We suspect this has led to considerable under-stimulation.

Discussion: H. Skjoldgaard, E. Goldschmidt, S. Ry Andersen

S. Vorn *Chamber depth before and after cataract extraction*

In a total of 80 patients with senile cataract the anterior eye chamber depth was measured skally by means of Haag Street's attachment II. The distance to the lower pupillary border preoperatively 2.59 ± 0.05 mm (mean \pm SEM). After cataract extraction it gradually raised to 3.33 ± 0.04 mm measured four months after the operation. The increase of depth was greatest in patients with a flat chamber and in elderly patients.

The central chamber depth gradually decreased after the operation (from 2.8 ± 0.1 preoperatively to 1.9 ± 0.13 mm four months postoperatively)

The number of vitreous prolapse cases rose from 68 to 88 per cent in four eyes. Possible causes were discussed

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Discussion S V Kessing A K Dreusler S Ry Andersen and Jens Edmund

Niels Vesti Nielsen and Per Nellesmann Sørensen Fluorescein angiography for borderline diabetes mellitus

Fluorescein angiography of the retina was performed in 54 persons with an abnormal 2 hour glucose tolerance test and in 23 controls with a normal glucose tolerance test. Microaneurysms were found in 48% of the series with a reduced glucose tolerance and late-phase microaneurysms in 43% of those having microaneurysms. Of the normal group 13 had microaneurysms but no leakage. Within both series ophthalmoscopy showed no microaneurysms and no signs of diabetic retinopathy. There was no relationship between the number of microaneurysms and the number of persons with abnormal glucose tolerance. On the other hand there was a correlation between inherited predisposition to diabetes mellitus and the occurrence of microaneurysms whereas smoking was of no importance. The results support the assumption that diabetic microangiopathy may already develop before diabetes mellitus becomes manifest.

Discussion H W Larsen M Warburg S Ry Andersen T Deckert (guest) E Gammelgaard Rosenberg E Cofsky Jensen

Jens Edmund Vitrectomy method - results - indications

Discussion N Ehlers H Skjoldgaard T Deckert (guest) E Dreusler E Gregersen P H H W Larsen S Ry Andersen P Brandstrup E Colischmidt

Eilif Gregersen J Pontoppidan and E Rindziunski Intra and inter-individual variations in atropine resistant residual accommodation

Accommodation is an extremely persistent reflex which has a marked memory effect. Therefore it is difficult if not impossible to abolish accommodation completely. This is reflected in the concept residual accommodation i.e. the accommodation remaining after application of a cycloplegic. The problems relating to residual accommodation are phenomena such as depth of field and tolerance of blur which have been reported to vary in a varying order of magnitude from one-quarter to one dioptre.

Among 25 amblyopic patients with esotropia who were treated in the course of fusion therapy with atropine 1% once daily into the dominant eye for about one year showed residual accommodation of ≥ 2 dioptres in the dominant eye. Thereafter the dose of atropine was increased to 1% twice daily up to 2% twice or thrice daily in an attempt to abolish the residual accommodation.

Summing up it must be emphasized that in some patients long term instillation of atropine can induce only partial not total cycloplegia. In such patients the atropine resistance

accommodation shows pronounced inter- and intra-individual variations with amplitudes from 1 to 10 dioptres as well as a sporadic and constant occurrence. The most marked atropine-induced residual accommodation was observed in two children who were "bookworms". Though at retinoscopy the patient is usually blinded by the light from the retinoscope and therefore does not use his residual accommodation during the retinoscopy procedure it is always possible to rule out a few or many dioptres of accommodation during retinoscopy or cycloplegia.

discussed in *extenso* in Transactions of the Third Meeting of the International Strabismology Association, Kyoto, Japan, 1978.

1978 on: P. B. Andersen, O. Hartnopp, V. Ehlers, E. Goldschmidt, V. D. Over

General Meeting

**October 1, 1977 in Copenhagen (Rigshospitalet)
with M. S. Norn in the Chair**

Reports were submitted from the Board, committees and commissioners.

The General Meeting requested the coming year to work out a report on visual and ocular care in Denmark.

The following were elected for the new Board: Eilf Gregersen (President), Erik Krogh, Mortensen, Erik Preisler and Erik Scherfing (Secretary).

472nd Meeting, December 3, 1977 in Copenhagen (Rigshospitalet)

A clinical pathological conference arranged by the University Institute of Ophthalmology.

Participants: Andersen, O. A. Jensen, Jan Linde, Prause.

Seven cases were elucidated: first by a brief clinical survey and thereafter by a pathological demonstration.

473rd Meeting, February 24, 1978 at Scania, Århus

Memorial Lecture

Professor G. Mackensen, Freiburg: *Microsurgical techniques and pathogenetic knowledge*

Professor Mackensen gave an inspiring memorial lecture on the development of microsurgical technique in ophthalmology. He stressed that after a trauma the surgery should be carried out as soon as possible and that complete reconstruction of the injured eye must be performed at the primary operation.

Postgraduate Course February 25 and 26 1978 at Scanticon Århus

Teachers Professor Benjamin Milder St. Louis Missouri Professor Robert M. L. Albany New York

In charge of the course Thomas Rosenberg

Subject Refraction

Basic book R. Reinecke & R. Hermann Refraction a Programmed Text Appleton-Crofts New York 1976

In 10 sessions followed by discussions: selected parts of the refraction problem elucidated in theory and in practice

Guest Lecture, February 25 1978 at Scanticon Århus

Professor Lalit P. Agarwal New Delhi India *Prevention of blindness in India.*

In an extremely thought provoking paper Professor Agarwal reviewed the total blindness in India and described the organization of the efforts to prevent blindness including those of DANIDA

474th Meeting April 15 1978 in Copenhagen (Rigshospitalet)

Mette Warburg *Congenital retinal malformations simulating retrolental fibroplasia.*

Congenital retinal non attachment (C N A) and falciform folds (C F F) may be the result of environmental influences in foetal life such as viral infections or X-radiation. Malformations may also occur in various chromosomal aberrations localized on chromosome 11. Non attachment may be found at the margin of colobomata of the choroid and genetic mutations may give rise to C F F or C N A.

Autosomal dominant mutations are rarely responsible for C F F or C N A. In reports of both types have been published. Autosomal recessive inheritance of malformations may be either confined to the eye or be part of complex syndromes. In events C F F and C N A may alternate in the two eyes of each affected individual and in eyes of affected members of a sibship. Several complex syndromes have been described: (1) Sarau's syndrome with C N A, osteoporosis and hypotonia (?) microcephaly, mental retardation and congenital C F F (2) the Meckel syndrome and (3) hydrocephalus, mental retardation and C N A.

There are also X linked complex syndromes with C N A or C F F i.e. Norrie's disease and the Bloch Sulzberger type of incontinentia pigmenti.

In all genetic disorders and particularly in autosomal recessive disorders parents appear as solitary cases and the C F F and C N A in such patients will show malformations indistinguishable from retrolental fibroplasia. In patients with these malformations the sporadic appearance of one of the syndromes listed above should be considered.

-osis of retinopathy of prematurity is made in full term infants who have been treated with oxygen

Discussion J Edmund S Ry Andersen H Skjoldgaard P Brandstrup V Wulfsberg E Jensen

Madsen *Experience of the transportable photocoagulator Log 2*

One year ago the Eye Department Roskilde acquired a transportable Xenon photocoagulator Log-2 from the firm Clinivet. The price was 50 300 Danish kroner +value added tax. This photocoagulator has been used for a number of different purposes and has proved efficient in practically all cases even in very fair patients with faint pigmentation of the iris and of the iris.

The only patients who could not be treated with the Xenon photocoagulator were those with a very small pupil less than 5-6 mm and patients with quite peripheral changes. The treatment factors were Power 2-6 for 0.2-1 sec. Diaphragm 4-4.5 for macular lesions and 5-6 for the retinal periphery.

There have been no complications of the treatments in particular no bleeding from the retinal vessels.

As a rule the patients were under retrobulbar anaesthesia but in a few the treatment could be performed without anaesthesia.

Among the last 20 patients treated with photocoagulation there were 12 diabetics some with simple and some with proliferative retinopathy.

These patients were treated partly with peripheral coagulation partly directly onto the retina. This applied in particular to the proliferative changes.

Seventeen patients with an exudative maculopathy the majority senile changes a few with serous retinitis. Our treatment was partly horseshoe-shaped coagulation at the edge of the oedematous area but later treatment was given centrally directly onto the oozing points.

Seven had venous thrombosis central vein occlusion or branch vein occlusion with macular changes. In such cases the coagulation was directed along the vessels in the involved areas.

Seven patients had retinal detachment retinal tears or retinoschisis. In these cases the treatment was partly at the edge of the retinoschisis and retinal detachment and partly directly onto the retinal tears.

In addition four cases of small or displaced pupils in aphakic eyes were treated with photocoagulation.

Conclusion The Log 2 photocoagulator is easy to operate and very handy. It is easily transported from one room to the other. It has sufficient power to afford reaction in light and in oedematous and somewhat detached retinal areas in senile maculopathy and in completely attached retinal detachments. It was also effective in the treatment of displaced irides.

Discussion E G Egersten O Nissen

Discussion E G Egersten O Nissen

Nellemann Sørensen Niels Vesti Nielsen and Knud Nørskov *Ocular retinoma A 15-year follow up*

Published 1978 in *Acta Ophthalmol. (Kbh)* 56: 363-372

Discussion E Gregersen O Nissen

■ Rv Andersen M Warburg F Geertinger H W Larsen M Willer Parving and S Vestermark *The deletion p 11 syndrome aniridia urogenital anomalies and mental deficiency*

The case of a 21 month-old girl the second child in a family without known congenital or malformation was reported

The child had bilateral aniridia cataract and mental deficiency Chromosome studies revealed a small interstitial deletion with band p12 and p13 on the short arm of chromosome 11 missing karyotype 46 XX del(11)(pter→p13 p11→qter)

Both parents and a half brother had normal karyotypes Thus the child's anomaly is considered to be a new mutation X-chromatin was present, but no Y was found and the child had female external genitalia The face was characterized by micrognathia low-set ears and mild coloboma of the nares The fundus of the right eye of normal appearance (the left one could not be visualized because of dense cataract) The girl died of pneumonia following measles

Microscopy disclosed bilateral benign gonadoblastomas in small gonadal streaks no sign of Wilms' tumour or of abnormal embryonal renal structures

Examination of both eyes revealed typical aniridia no iris muscles and no anterior cleavage of the lens Both lenses were small and cataractous The pigment epithelium of the retina was disintegrated in places and in the right eye some of the peripheral retinal vessels were occluded Small retinal strands proliferated into the vitreous in this area No foveal or papillo-macular bundle could be identified

In the literature three patients with deletion of the short arm of chromosome 11 and mental deficiency and genital malformation have been described by Francke et al (1966) who have reported a Wilms' tumour Ladd et al have described a patient with aniridia a Wilms' tumour and interstitial deletion of the short arm of chromosome 8 In our opinion the most likely from chromosome 11

One patient with aniridia gonadoblastoma and genital malformation but without chromosomal studies has been reported by Gracia et al and DiGeorge et al described a patient with gonadoblastoma aniridia and Wilms' tumour

We feel that the cases reported so far including our own are characteristic of a syndrome with partial deletion of the short arm of chromosome 11 aniridia and/or genital dysmorphogenesis

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- Gracia R Nieto J A Nistal M Iturriza R Lledo G Barrio R & Lema E et al Pediat 1976 9 suppl 8 19-24
- Ladd R Atkins L Littlefield J Neurath P & Marumitsu A M Science 1971 171 784-787

Discussion P Brøndstrup E Krogh

Thygesen Per Reersted Hans Fledelius and Leif Corydon Astigmatism after cataract extraction Late results after corneal incision and corneo scleral incision respectively published 1979 in Acta ophthalm. (Kbh) 57 243-251

Discussion A A Dreusler V Bulow M Hense E Gregersen S Barner

Norn Spheroidal degeneration of the cornea and conjunctiva published 1978 and 1979 in Acta ophthalm. (Kbh) 56 551-562 and 57 96-103

Discussion V Dreyer S Ry Andersen A A Dreusler O A Jensen P Alsbræk E Skeller A Jørgensen V Willumsen P Brøndstrup

Prause Antiproteases and collagenase in human lacrimal fluid Method of local problems and a modification of a new micro-immunoelectrophoretic method

After a brief presentation of the accepted mode of in vivo destruction of corneal stroma living tissue collagenases the more specific balance between the serum antiproteases fibrinolysin alpha 1 antitrypsin alpha 1 antichymotrypsin and alpha 2 macroglobulin on the one hand polymorphonuclear leukocyte collagenase (PMNL-collagenase) on the other was described by this method the author avoided the many errors due to organ culture by measuring protease and the PMNL-collagenase in lacrimal fluid from patients with corneal ulcers as PMNL-collagenase is found only in minute amounts in lacrimal fluid a micromodification of the electroimmunoassay of Laurell has been elaborated (1) Using this assay and a rabbit anti-human leukocyte collagenase (2) it has been possible to detect the named balance in lacrimal fluid from patients with corneal ulcers

The material is being prepared for publication in an extended form in Acta Ophthalmologica

References

Præuse J L Synstrom H Noren O & Josefsson L (1978) A micromodification of the electroimmunoassay Anat Biochem 85 564-571
 Olsson A & Olsson (1973) The neutral proteases of human granulocytes Eur J Biochem 36 473-481

Discussion

Gregersen and J U Prause Corneal perforation after the Elmore pill published in Acta ophthalm. (Kbh.)

Discussion V Dreyer M Waabug S Ry Andersen V Willumsen S Alsbræk J Thygesen K A Jørgensen J Bøberg An

Brøndstrup Open angle glaucoma burden of patient and doctor

Thom J Zimmermann Louisiana State University (invited as a guest through the pharmaceutical company Merck Sharpe & Dohme)

Thom J Linné A new glaucoma medication

Herbert E. Kaufman Louisiana State University (invited as a guest by pharmaceutical company Merck Sharpe & Dohme)

Timolol maleate A new glaucoma medication.

Niels Vesti Nielsen *Timolol for the treatment of intraocular hypertension (beta-blocker)*

Published 1978 in Acta ophthalmol (Kbh) 56: 504-509

E. Gregersen and S. Kessing *Short term effect of timolol as a supplementary medication of glaucoma*

Discussion of the last five papers with contributions concerning the long-term indication for timolol therapy was recorded on a tape by Merck, Sharpe & Dohme

TRANSACTIONS OF THE SWEDISH OPHTHALMOLOGICAL SOCIETY 1978

EDITED BY

GUNNAR LENNERSTRAND

Meeting in Huddinge June 2-3 1978 General Session

oller & P Enoksson *Congenital ocular motor apraxia*

Iolm *A new high power microscope for studies of the anterior segment of the human eye*
Standard corneal microscopes do not permit a higher magnification than 25-40x. They
herefore of limited use in studies of cellular structures. Such studies however have
me increasingly interesting also in routine clinical work. It is \equiv g important to know the
lution of the endothelial cell layer of the cornea before performing certain intraocular
scal interventions.

struments for studies of the corneal endothelium have been available for some time but
require physical contact with the cornea and do not enable studies of deeper structures.
new high power microscope attachment has been developed. It can be used with any
 \equiv Strent type corneal microscope. It utilizes regular slit illumination and does not require
act with the eye. The focus can be varied from the surface of the cornea to the anterior
ace of the lens. The magnification can be varied between 100 and 250x.
odifications for use on Zeiss slit lamps and for photography are being developed.
With this instrument called EMS I studies can be made of \equiv corneal epithelial and
othelial cells, lens capsular cells, capsular exfoliations etc.

Germanis *Some ophthalmological observations on children with reading difficulties*
omplete ophthalmologic examination including orthoptic investigation was done on 110
ool children who were referred to an ophthalmologist because of delayed reading ability.
of them were given remedial teaching. No attempts were made \equiv differentiate the group
o primary and secondary reading retardation.
binocular disorders causing subjective complaints at the reading distance were looked for.
paired binocularity was revealed in 30% of the children and half of them complained of
henopia, blurring of vision or diplopia. The binocularity disorders present and their

relative incidences were exophoria 9% esophoria 4% tropia 4% fusional defects accommodative insufficiency 8%. From 6 prism dioptres exophoria to 4 prism dioptres esophoria was considered as orthophoria. Most of the heterophorias present were hyperphorias.

Most prone to subjective complaints were exophorias accompanied by convergence deficiency and the cases with accommodative insufficiency. The latter was characteristically changing and for the age remote near point of accommodation. In half of the cases poor accommodation was associated with hyperopia and low grade esophoria. They helped with glass correction. In the other half the accommodative insufficiency was accompanied by convergence deficiency and most case histories revealed emotional factors or subclinical systemic disease.

The importance of measuring the near point of accommodation as well as the near point convergence is stressed when dealing with problems at the reading distance.

M Cjotterberg *Photocoagulation of Macular Diseases*

Macular lesions complicated by fluid leakage or neovascularisation are sometimes amenable to treatment by photocoagulation. The indications for treatment of the following diseases are discussed and slides of typical cases presented.

1 Central serous retinopathy. The main factors when considering treatment are the complaints of the patient and the location of the leak. Coagulation of a peripheral periphery of the macula is a very safe procedure which significantly shortens the duration.

2 Exudative senile macular degeneration. Detachment of the pigment epithelium is sometimes the initial event and may be treated. The rationale of the treatment is to prevent the development of cicatricial stages.

3 Branch vein occlusion. Some patients develop macular oedema which may lead to degenerations. The hypoxia may cause neovascularisation. These cases with an unfavourable prognosis are candidates for treatment.

4 Diabetic maculopathy. Treatment of patients with oedema and/or neovascularisation is often beneficial.

The main problem when considering photocoagulation treatment of macular lesions is how to treat but when to treat. The unpredictable course of the involuntarily degenerative lack of controlled studies as well as the palliative nature of the treatment are all factors which render the assessment of the therapeutical results difficult.

I Aringer *Chlamydia trachomatis in iritis and acute eye infections.*

This study was initiated from the connection between iritis and ankylosing spondylitis. It was started in 1975 with a study of the presence of chlamydial trachomatis in iritis. Samples were taken from the conjunctiva of 12 patients with acute iritis. Chlamydia was demonstrated in two of the patients. Both were healthy except for their iritis.

Serum samples from 38 patients with acute iritis were sent for analysis to the Erik Löfdahl Virol lab Gothenburg. Group specific chlamydia antibodies were demonstrated in 10 patients (24%). Analysis of serum from controls that has been made at the same laboratory has shown group specific chlamydia antibodies in 13% of the samples. Of the 38 patients 10 had ankylosing spondylitis and two prostatitis but none of them were positive. Seven had previous history of urinary or genital tract infection four of them were positive for chlamydia antibodies. Five of the 9 patients that were positive were healthy except for their iritis. Samples from the conjunctiva were also taken but they did not survive the transport.

Smears were also taken from the conjunctiva of 102 patients with a wide variety of acute infections. They were analysed by doc G-O Kindmark and dr Rolf Zimmerman, Virol. Natl. bact. lab. Stockholm. In five of these patients chlamydia trachomatis could be detected from sample from conjunctiva. One of them had recurrent dacryocystitis, two had iritis, one had acute and one chronic conjunctivitis. One of the chalazion patients healed spontaneously, whereas the other four patients had not improved during 2-3 weeks observation time. They recovered quickly when treated with tetracycline.

Conclusions: Chlamydia trachomatis has been shown to be present in various types of acute eye infections. In this material, patients from which chlamydia had been cultivated had shown a clear tendency toward a prolonged illness. The serum samples from the patients with iritis showed a higher incidence of chlamydia antibodies compared to controls. This higher incidence cannot be explained by a history of ankylosing spondylitis or prostatitis.

Symposium The Eye in Diabetes

Director B Zetterstrom-La pte

Members S Stenkula, M Gyllenberg, P Algvere, S Blomdahl, J Christiansson, V Maen, & B Philipsson, M Pandolfi.

1. Gyllenberg: *Altered blood flow properties in the diabetic retina*

Review over the current hypotheses behind retinal hypoxia was presented. Among the multiple contributory factors suggested, alterations in blood flow properties are stressed.

Algvere & B Kornacki: *Fluorescein angiography of the iris in diabetic eyes*
Ophthalmologica 36: 803-816, 1978.

Blomdahl & M Gyllenberg: *The human electroretinogram follows argon photocoagulation*

Christiansson: *Diabetic retinopathy and pregnancy*

A total of 94 763 obstetric patients at the Central Hospital in Kristianstad, 78 diabetics detected. All except five of them were checked as to the influence of pregnancy upon eventual diabetic retinopathy. Thirty-five patients (48%) with an average diabetic duration of 15-16 years showed ophthalmoscopic signs of diabetic retinopathy. In seven of these the retinopathy progressed during pregnancy. Ten patients (14%) had severe proliferative retinopathy, of whom five actually developed this condition during pregnancy. In these 10 cases, induced abortion was performed in two cases. The remaining eight patients gave birth to six surviving children. After delivery, some reversion of proliferative retinopathy was observed in all cases, often with a remarkable reduction of intra-retinal exudate tufts.

Argon photocoagulation treatment by Scatter Xenon photocoagulation seemed of value in one case of severe elevated vascular neoformation. The left eye was treated before pregnancy and four years later. The Snellen visual acuity was 0.7. The non-treated eye showed a massive vitreous haemorrhage and a Snellen visual acuity of 0.1.

In conclusion the incidence of diabetes in pregnant women is steadily increasing 0.3% in the present population. The frequency of severe retinopathy is also increasing putting a strong demand on ophthalmic services.

Preventive photocoagulation should seriously be considered whenever progression towards a proliferative stage that will endanger vision.

N. Maren & G. Jøtterberg *Diabetic retinopathy and cigarette smoking*

S. Boos *Sensitivity to dazzling light of diabetics during driving in darkness*

In order to ascertain whether persons with diabetes mellitus have impaired vision in the dark, the readaptation time (RAT) after dazzling light has been measured. They were compared with those of non-diabetics.

Method. The persons taking part in the experiment were made dark adapted in the testfield with the luminance of 0.1 cd/m². A round object with the diameter of 10 cm was moving forward and backward horizontally. A dazzling light of 0.3 lux was on for 1, 4 and 16 seconds. It subtended an angle of pursuit eye movements.

Results. All diabetics fulfilled the requirements for a Swedish driving licence and had a visual acuity of 1.0. Visual acuity in the dark (luminance 0.1 cd/m²) was 0.4 for diabetics and 0.6 for the non-diabetics. The diabetics with retinopathy showed a longer RAT. After a dazzling light of 1 second RAT was 2.7 second for the diabetics and 1.0 second for the normal group.

After a dazzling light of 4 and 16 second respectively the values were 2.0 second for the diabetics and 1.0 second for the non-diabetics.

Conclusion. This preliminary study shows that the diabetics with retinopathy have impaired visual functions in darkness when driving a car by night, even if they have good visual acuity.

P. Algvere *Vitreotomy and excision of preretinal proliferations in diabetic sequelae*
To be Published 1979 in *Acta ophthalmologica* 57: 530-549

M. Pandolfi & S. Stenkula *Induction of fibrin dissolution in the vitreous body*

Injection of urokinase (a fibrinolytic activator derived from human urine) has been proposed as a treatment of intraocular haemorrhages. On the basis of a review of the literature and of own experiments both on animals and in vitro the authors draw the following conclusions:

1) The urokinase used hitherto can be purified further and has of course been given in much higher doses.

2) The optimal dose to cause a maximum fibrinolysis in the eye is probably smaller than that used until now.

3) The therapeutic effect if any of such a treatment is due to the fibrinolytic activity of the plasmin formed.

Meeting in Sundsvall September 29–30 1978

osium Visual field testing in glaucoma

rator L. Frisen.

members M Lundström P Enoksson H Brynke C Holm

sen The pathophysiology of visual field defects in glaucoma

sen The tangent screen examination

General Session

Anderson & J Sjostrand The function of the macula following retinal detachment in olding cula

aim of the present investigation was to evaluate different macular functions during following operation for retinal detachment involving the macula

hods Among all retinal detachment patients during a two and a half year period its (7) were selected meeting the following criteria visual acuity > 0.3 clear ocular absence of diabetes or previous macular disease The following parameters were d postoperatively (2 months to 3 years following operation) Visual acuity contrast ivity grating acuity micropsia and metamorphopsia.

and sensitivity was measured using a TV monitor displaying a vertical sine wave grating m of variable spatial frequency and contrast (Sjostrand & Frisen Acta ophthal. (Kbh) 55 14 1977) Test distance was 5 m

acuity is a measure of the capacity to resolve a grating pattern A vertical sine wave g pattern was displayed with the same equipment as used for the contrast sensitivity minations Test distance was 7.9 m and maximal contrast was used

cropsia Six out of the seven patients studied had micropsia The degree of micropsia was ified with an instrument constructed by L. Frisen

amorphopsia Five of the patients had metamorphopsia It was tested by using the ler's grid

is Contrast sensitivity In a group of patients examined more than a year following achment an abnormal contrast sensitivity function could be observed in two age groups high and intermediate frequency ranges were affected predominantly Currently we are ring the spatial processing preoperatively and during the recovery phase Preoperati elv in the early phase following operation the contrast sensitivity is impaired over the whole uency range with a successive improvement during recovery

ating acuity In the patients examined the grating acuity showed a tendency to be higher atients with a recovery period of more than two years No correlation between time of perative macular detachment and postoperative grating acuity was found in the patients ied However in this retrospective group of patients it was difficult to define the time of perative detachment

micropsia The micropsia ratio (ratio between size of the image in the eye with retinal tachment and fellow eye respectively) was 0.71–0.91 Some of the patients had a marked rpsia even a long time after operation The micropsia was not correlated in the visual iv The cause of the micropsia is presumably a changed distribution of photoreceptors in persistent macular oedema or proliferation of glial tissue

Metamorphopsia A varying degree of metamorphopsia was observed. In some cases correlation between the results of the Amsler's test and the funduscopic changes was found.

Conclusion The postoperative macular function after reattachment of the retina was abnormal for a long period of time and various functions can be differently affected.

P. Algvere *Pars plana vitrectomy in amyloidosis. Report of a case*

Primary systemic amyloidosis with peripheral neuropathy and ischemic heart disease was found in a male aged 66 and verified by a rectal biopsy. He became blind on both eyes (only light projection) due to dense vitreous opacifications.

An intracapsular cataract extraction was performed and 4 weeks later a pars vitrectomy (using Klotz's vitreous stripper) enabled removal of the amyloid material so that the optical transparency of the eye was restored. There was a rapid postoperative recovery and in 2 weeks the visual acuity was 0.5 (aphakic correction) and in 8 weeks 1.0.

At a follow up examination 10 months later the visual acuity was 1.0, the eye free of inflammation and the vitreous space was clear, there being no new formation of amyloid material in the vitreous space. The fellow eye was still blind.

Pars plana vitrectomy yields good results in amyloid vitreous opacification and is in several aspects superior to the open sky technique used earlier.

L. Frisen *Micropsia and macular oedema*

R. A. Ronnerstam *Occurrence of Chlamydia Trachomatis associated with external eye disease. A preliminary report*

The occurrence of Chlamydia Trachomatis has been investigated during a period of 7 months in 101 patients treated at the department of Ophthalmology in Malmö. In 10 patients with acute conjunctivitis and keratoconjunctivitis. Six of these patients were newborn children. In four adults in fertile age with serious keratoconjunctivitis and in 10 newborn children with conjunctivitis.

These findings prompted a five months investigation of further patients with symptoms of external ocular inflammation irrespective of age and seriousness of disease. In this clinical material Chlamydia was isolated only in one adult and two children. During the same period search for Chlamydia was made in all newborn children of this time and in their mothers. Out of 600 infants one was found to have Chlamydia in the conjunctiva and in 10 mothers were found to have Chlamydia in the cervix uteri.

During these seven months 28 newborn infants with conjunctivitis were examined in the ophthalmic department. In five of these the isolation of Chlamydia was positive. This is a significantly higher frequency compared with 1/600.

Chlamydia isolation might be of clinical importance since Chlamydia is known to cause pneumonia and other infections in newborn children. Furthermore it may cause disease in the mother.

In Malmö we treat newborns with Chlamydia conjunctivitis with oral erythromycin together with chloramphenicol or sulphonamides locally. Also the mothers of such children receive erythromycin while all other adults are given doxycycline. All patients have responded well to the treatment.

dergh A Bill P Kaufman & E Lutjen Drecoll *Effects of Cytochalasin B and the anterior segment of the eye*

cameral infusion of Cytochalasin Bor EDTA (ethylenediaminetetracetate) in the living eye eliminates about 3/4 of the total resistance to aqueous humor outflow morphologically Cytochalasin B causes a relaxation of the cells in the cribriform meshwork of the extracellular material and ruptures of the inner wall of Schlemm's canal The endothelium and ciliary body are also affected (*Invest Ophthalmol Vis Sci* 17 718-734)

A breaks up the cell junctions of the trabecular meshwork with loss of the extracellular material in the cribriform area The inner wall of Schlemm's canal balloons into the lumen or tears In the corneal endothelium the apical cell junctions separate physiological effects are reversible within 1-2 h The morphological effects of cytochalasin B are reversible within 7 days Studies of the healing process after EDTA are in progress

Attrell & M Pandolfi *Propranolol versus acetazolamide: A doublemasked study concerning intraocular pressure and systemic blood pressure*

Walinder E B Hakansson & M Lindberg *Blocadren (Timolol) ophthalmic solution in the treatment of simple and exfoliative glaucoma: a short term study*

Blocadren (timolol maleate) is a beta blocking agent which as an ophthalmic solution has a hypotensive effect on open angle glaucoma equal to 4% pilocarpine It has no serious side-effects (Symposium on Glaucoma XXIII Int Congress of Ophthalmology Kyoto 1978)

1/ Previously untreated chronic open angle glaucoma

1/ An applanation pressure curve was performed 1-2 days before treatment was started with 0.5% Blocadren eye drops x 2 If intraocular pressure (IOP) was not controlled (mmHg) therapy was changed to 0.5% Blocadren x 2 and then addition of 2-4% pilocarpine acetazolamide or the eye was operated upon

The study included 21 eyes in 19 patients mean age 67 years There were 5 simple and 16 exfoliative glaucoma with an average IOP before treatment of 30 and 32 mmHg respectively Fifteen eyes had obvious visual field defects and glaucomatous changes in the optic disc the rest had suspected changes of the same kind Blocadren caused a mean reduction of IOP of about 40% in the simple glaucoma during a period of 3 months

In the exfoliative glaucoma (16) had a reduction of IOP of about 40% during the first days in the hypotensive effect of Blocadren decreased and only 3 eyes were controlled during 3 months Nine eyes required additional 2-4% pilocarpine and one eye also required acetazolamide to be satisfactorily controlled during a period of 3 months Operations were performed in three eyes

Blocadren had a doubtful hypotensive effect in a patient on propranolol 10 mg x 3 orally there was a slight reduction of IOP in a patient on alprenolol 0.1 g x 2

Blocadren caused no side-effects

Conclusions In this short term study Blocadren alone controlled IOP in simple glaucoma exfoliative glaucoma often required additional therapy

Meeting in Stockholm November 30 – December 1 1978

General Session

G Lennerstrand *Structure of extraocular muscle in Siamese cats and domestic cats – unilateral lid closure*

We have previously reported differences in eye muscle functions between normal cats and cats with binocular defects (Siamese cats and domestic cats with microphthalmia from age 2–3 weeks). Speed of muscle contraction and fatigue resistance was reduced in the latter group. The differences were most marked between normal and Siamese cats. For correlates to the functional changes were explored in histochemical experiments on eye muscles that were examined physiologically. Transversal muscle sections were stained for myosine ATPase and succinic dehydrogenase. Type I and II fibers were identified. Proportions and distributions of the two fiber types were the same in all muscles. Succinic dehydrogenase content of the fiber populations was not significantly different of the groups of cats.

However, the total cross sectional area of the muscle was smaller in Siamese and lid-closed cats than in normal cats, due to a general decrease in size of both type I and II fibers. The number of capillaries per muscle fiber was reduced in the Siamese and lid-closed animals in comparison with normal cats.

It is suggested that the decreased speed of contraction is correlated with the reduced fiber size, and the lowered fatigue resistance with the decreased capillary density in Siamese and lid sutured animals. These changes might represent muscular adaptation to lack of binocular vision and to reduced demands for fusional vergence movements.

M Pandolfi & E Lantz *Keratokinase, the urokinase like plasminogen activator of the eye*

P Algvere & A Bill *Effects of lens removal and vitrectomy on drainage of particles from the vitreous space*

The movements of plastic microspheres (7–10 μ m in diameter) and radiolabelled red cells from the vitreous to the aqueous compartment were studied in vitrectomized and non-vitrectomized phakic and aphakic eyes of 18 rabbits. The particles and red cells (in suspension) were injected into the centre of the vitreous space, observed by microscopy and studied with histology and autoradiography.

In phakic eyes the particles and red cells were unable to gain access to the anterior chamber, irrespective of whether a vitrectomy was performed or not. Following lens removal and breaking of the anterior vitreous border, microspheres and red cells reached the anterior chamber and chamber angle within 2–10 days. A combined lensectomy and vitrectomy resulted in rapid clearance of the vitreous space, and large amounts of particles were found in the inferior chamber angle within a few days.

The experiments indicate that the most effective clearing of blood from the vitreous occurs after a combined lensectomy and vitrectomy. Following a vitrectomy alone, the anterior vitreous border still prevent particles from entering the aqueous compartment.

L Öhman, L Berggren, R Hahnenberger & E M Johansson *Transfer of free steroids (17 β -oestradiol and progesterone) from the eye and nose to plasma and cerebrospinal fluid*

The free steroids norethandrone, progesterone and 17 β -oestradiol have been given as eye-drops or been given as drops or spray in the nose of rhesus monkeys and

The resorption to blood and cerebrospinal fluid. The administered dose has been 1/2 mg steroid (1 drop in each eye or nostril of 1/2% or 0.5% suspension or solution only). Blood and cerebrospinal fluid samples have been taken before the administration of the drops and frequently during one h. The steroids have been analysed by immunoassay.

The free steroids are rapidly resorbed into the plasma, e.g. in a couple of min. the rising levels of hormones increase several times. The resorption is much better for the steroid in solution than in suspension. Maximum levels of hormones in plasma are seen after 15-30 min. The levels of hormones in the cerebrospinal fluid are generally low.

The three steroids are rapidly resorbed to plasma after ocular or nasal administration. The levels of hormone in the cerebrospinal fluid are generally low.

Schachtmeister & J. Dowling. Microelectrode study of the oscillatory potentials of the retina (ERG) of the mudpuppy. *Ophthalmol Vis Sci* 1979; 17: 1176-1189.

Sjöstrand & I. Burså. Contrast sensitivity in amblyopia.

Amblyopia due to strabismus or anisometropia is in most cases treatable if treated at an early age. With correction of refraction errors in combination with patching visual acuity is improved.

The aim with our study was to analyze the contrast sensitivity function in patients (age 5-15 years) with amblyopia due to strabismus or anisometropia with the following questions: Are there any differences in contrast sensitivity functions between the two types of amblyopia? Can changes in the contrast sensitivity function be documented in children during treatment?

The contrast sensitivity for sinusoidal gratings of varying contrast and frequency was determined monocularly by raising contrast from a subthreshold setting until a grating pattern was faintly seen. With plaiding technique measurements could be done down to an age of 5 years with good reproducibility.

Children (age 5-9 years) were tested before and following treatment. The contrast sensitivity curves of the non amblyopic eyes were within or slightly lower than those measured in adult normal subjects in an earlier study (J. Sjöstrand & L. Frisén. Contrast sensitivity in macular disease. A preliminary report. *Acta Ophthalmol* (Abh.) 55: 307-314, 1977).

In strabismic amblyopia (visual acuity 0.1-0.3) before treatment the contrast sensitivity for intermediary and low spatial frequencies was similar to that measured in the contralateral amblyopic eye and no effect of patching could be demonstrated in these spatial ranges in terms of improvement of visual acuity.

In amblyopia due to anisometropia (visual acuity 0.1-0.2) the contrast sensitivity curves were drastically depressed before treatment and during patching treatment an improvement was found in both contrast sensitivity and visual acuity.

In young adults (age 15-31 years) with amblyopia (visual acuity 0.05-0.2) abnormal contrast sensitivity was demonstrated in both strabismic and anisometropic amblyopia. However, the changes were more marked in anisometropic amblyopia.

Conclusion Contrast sensitivity studies can be carried out down to an age of 2 years. A determination of contrast sensitivity gives a more general information concerning visual function than tests with sinusoidal gratings displayed on a TV screen at an angle at the eye of 15°. A marked impairment over the whole frequency range was demonstrated in amblyopia and anisometropia whereas no changes were found in the intermediary and low frequency range in strabismus before or following treatment. During successful patching treatment of anisometropic amblyopia an improvement of contrast sensitivity was documented.

K. Olsson & A. Hedin *A better visual acuity test chart?*

The readability of 23 letters were studied in 9 subjects with normal visual acuity. On the basis of the result a test chart was constructed with geometric size progression.

G. Lennerstrand *Adjustable sutures in strabismus surgery*

The technique of adjustable sutures in squint surgery (reviewed by Jampolsky *Trans Acad Ophthalmol Otolaryng* 79:704, 1975) has been used in eleven cases of strabismus with incomitancy due to paralysis, contracture or scar structure.

After premedication with atropine, general anaesthesia was induced with halothane or fentanyl-N₂O. The muscle to be adjusted was secured with 6-0 PDS suture. One end was attached to the sclera, but the sutures were not tied. A slip knot was applied to the other suture ends, preventing the muscle to retract beyond the desired position. Sutures were placed between the original muscle insertion and the limbus.

When the patient had fully recovered from anaesthesia, which occurred immediately postoperatively, eye alignment was tested in different directions of gaze. If necessary, the position was adjusted under topical anaesthesia by sliding the slip knot the desired amount, thereby changing the length of the muscle sutures. The suture ends were secured by the sliding knot and trimmed.

Suture adjustment was performed postoperatively in nine of the patients. 6 muscles were moved. A 8 year-old child failed to cooperate, but adjustment was possible in another child, 10 years-of age. The rest of the patients were between 16 and 66 years old.

The possibility of correcting eye position by suture adjustment in the immediate postoperative period is a valuable supplement in the surgical management of selected strabismus. It is questionable, however, whether this procedure should be attempted in children.

E. Wold & E. M. Møller *Pregnancy and diabetic retinopathy*

I. Gislason, A. Alm, S. Stenkula, F. Wold & P. E. Wålinder *Correlation between the thickness of the retina and visual evoked potentials*

B. Philipson & A. Philipson *Postoperative endophthalmitis*

Endophthalmitis is one of the most serious complications following ophthalmic surgery. The complication has attracted much attention recently because 1) it appears that the incidence of postoperative endophthalmitis in Sweden has increased over the last few years, 2) increased knowledge of pharmacokinetics of antibiotics has substantially improved the possibility of successful treatment.

A fundamental principle in the treatment of endophthalmitis is to reach therapeutically adequate levels of antibiotic rapidly in the vitreous. A great variety of bacterial species, gram positive as well as gram negative, can cause endophthalmitis. In recent years it has become evident that *Staph. epidermidis* is a frequent cause of endophthalmitis which may appear one or two weeks following ophthalmic surgery. As antibiotic therapy must be given immediately, results from bacteriologic cultures are not at hand. Thus, a wide bacterial spectrum must be covered. Suitable antibiotics for initial treatment are gentamicin and penicillin G. If the logical agent is suspected to be beta-lactamase producing staphylococci, gentamicin and isoxyl penicillin are a better combination.

Different routes of administration can be used. The fastest way to achieve a high level of antibiotic in the vitreous is to inject the drug into the site of infection, i.e. intravitreally. However, several studies confirm that administration of the antibiotic by injection on subconjunctally or subtenonally is also highly effective. Such administration should always be coupled with simultaneous administration of the same antibiotics given parenterally. Intravitreal administration of these antibiotics is not acceptable as sufficient levels of antibiotics cannot be reached in the eye. Treatment should be supplemented with oral steroids.

In the case of late postoperative endophthalmitis following cataract surgery has been treated at Eye Clinic at the Karolinska Hospital. Culture from the vitreous revealed *Staph. epidermidis*. In this case therapy was given as follows: An intravitreal injection of penicillin G 3000 IU was given upon admission. Simultaneously, gentamicin 40 mg and penicillin G 3000 IU were injected subtenonally, and these injections were repeated on the second and third day. Systemic therapy was given with gentamicin 90 mg intramuscularly t.i.d. and penicillin G 5 million IU q. 4 h intravenously. Prednisolone 60 mg was given daily by mouth. Soon as culture results were at hand, penicillin G was exchanged for cloxacillin 2 g q. 4 h. Intensive antibiotic therapy was given for 12 days. This therapy gave a total remission of the endophthalmitis. However, a reduced visual acuity remained.

Rosengren: The effect of vitreous currents on different types of retinal holes and tears.

The influence of vitreous currents on different kinds of retinal holes and tears and their tendency to promote detachments is not clearly established.

In model experiments a container with an internal diameter of 29 mm has been used. It is placed on a stand which permitted rotation of the container and even attachment to a pump. The inner wall of the container was fitted with circular elevations onto which a plastic membrane with an aperture (diameter 2 mm) was attached.

When the container is filled with solution of water and suspended particles, the plastic membrane symbolizing the retina will be surrounded by the suspension. Consequently, when the container is rotated, it is possible (with the aid of a slitlamp) to observe currents on both sides of the membrane and passage through the aperture.

When the membrane is parallel to the direction of the current, rotation will not cause any flow through the aperture. Passage will, on the other hand, occur if the membrane makes an angle with the direction of the current. The same thing may also happen if the aperture in the membrane is distorted.

Structures on the edge of the aperture rising above the membrane cause irregular currents and consequently friction which has to result in traction on the above mentioned structures. In vivo this will cause traction on structures on the edge of a retinal tear and thus lead to result in detachment.

The diameter of the aperture is also of greatest importance. Diameters of 2 mm, 1 mm and

0.5 mm were compared. When these are placed on a line on a sloping membrane and through the aperture forms easily when the diameter is 3 mm. This is more uncomfortable of the 1 mm diameter and no current passes through the 0.5 mm aperture. In vivo conditions this means that small retinal holes hardly permit passage of currents.

When elevation of the retina is present another factor will be involved the inter-subretinal fluid. This explains the quick fluctuations in the extent of the elevation.

Ilmarinen Lecture

Professor Rudolph Wirtmer (Zurich) *Ophthalmology of Uveitis*

The clinical picture of various forms of uveitis was described: anterior uveitis (fibrinous, iritis, chronic anterior uveitis in children, iridocyclitis, heterochromia, chromocyclitis), posterior uveitis (central choroiditis, choroiditis, juxtapapillary seropigment and dissemination) and panuveitis (in Bechet's disease and in hereditary forms). It was emphasized that even if each form is easily identifiable this seldom gives information about the aetiology of the inflammation. The treatment in most cases consists of steroid therapy systemically. The use of immunosuppressive and cytostatic drugs (such as cyclophosphamide and Natulan) was considered a valuable alternative or complementary treatment in cases of chronic cyclitis (in children) and of panuveitis.

With regard to laboratory findings in uveitis it was pointed out that 1) bacterial invasion of the ocular tracts is very rare, 2) serological tests (for toxoplasmosis, tuberculosis, streptococcal and viral infections) can never alone give a definite proof of the aetiology, 3) aqueous humour studies have shown that antibodies are produced locally in the uveitis, 4) the percentage of positive findings in uveitis has been very low, 5) electron microscopy of iris biopsies have demonstrated immunologically competent cells in cases of intraocular HLA system studies have shown a close connection between antigen presentation in patients with acute fibrinous iritis and the occurrence of HLA B27 antigens and in Bechet's disease and HLA B2 antigens, 6) the level of complement components C3 and C4 in aqueous humour was lowered in anterior uveitis while that of C4 and IgA was elevated.

It was concluded that uveitis remains a mysterious disease. Infections may be important but can very rarely be proven. Immunological phenomena do occur as shown by serological studies on the aqueous humour. Electron microscopy of iris and detection of IgA, C3 and C4 in the HLA system reflects only the terrain upon which uveitis may develop.

JUDICIA DE NOVIS LIBRIS

ssor Maurilio Pandolfi M D Malmö Sweden Hemorrhages in Ophthalmology -- A Hemostatic Approach Georg Thieme Publishers Stuttgart Price DM 48 -- 84 pages 61 figures 17 tables

ocular haemorrhages whether spontaneous traumatic due to eye diseases or following surgical intervention will always constitute a problem to ophthalmologists Dr Pandolfi's after an introductory chapter on the mechanism regulating the haemostasis treats of ocular haemorrhages and haemostasis The author groups the intraocular haemorrhages according to their sites in the eye beginning his description with haemorrhages and fibrinous exudation in the anterior segment Then follows sections concerned with haemorrhages in the vitreous body conjunctiva retina and choroid A fairly large section concentrates on haemorrhages in relation to ophthalmic surgery and the measures to be taken to avoid pre- and postoperative haemorrhages e.g. in patients with a haemostatic defect In an appendix an account is given of the haemostasis in the presence of retinal thrombo-embolism and retinitis proliferans The author attempts in each section to throw some light on the various factors that may influence the pathogenesis and the course of haemorrhages on the eye In addition suggestions for prophylaxis and therapy are reviewed The book gives a clear and easily read account of a very special subject It is highly commendable

S. Fajersjö

Barsewisch B Perinatal Retinal Haemorrhage Morphology Aetiology and Significance 184 pages 64 figures 13 plates 9 tables Springer Verlag Berlin Heidelberg New York 1979 ISBN 3 540-0167 X Cloth Price DM 58 US Dollar \$1 90

This is a valuable comprehensive monograph dealing deeply An extensive literature has been collected and commented on in this book describing well-established morphological variations histologic studies and last but not least propounding a conclusive view regarding the prognosis

Ever since the infancy of ophthalmology numerous colleagues have with surprise and even horror noted the high incidence of extensive retinal haemorrhage of new-born babies (in the newer's opinion in not less than 50 per cent) This seems particularly surprising compared with analogous extravasations of blood in adults The authors have wondered at the generally very rapid absorption of the blood They have found it difficult to believe that macular oedemas may be harmless having on the contrary judged them to be responsible for otherwise unexplainable amblyopia

The author's series comprises 400 cases subjected to a careful morphological classification histologic examination where such could be carried through study of a relationship if any between the course of the delivery and possible medical intervention in this

Follow up examinations have shown such haemorrhage to be harmless In very rare cases only it may be suspected to be the cause of impaired function

With the publication of Barsewisch's investigations the last word should in the reviewer's opinion have been said on this subject

P. B. Öndrup

M. Ruben Contact Lens Practice — published by Bailliere Tindall London 1979, available in German

The German edition is published by Gustav Fischer Verlag Stuttgart — 1981 translated by Dr. Hans Walter Roth the university clinic in Ulm

The two editions are identical as to the illustrations and very similar in the text. Dr. Walter Roth has excellently translated the many Anglo-Saxon technical terms into German which however is hardly of any help to those who are accustomed to these terms.

The book provides the practitioner with a basic knowledge of contact lens fitting, the physiology of the eye and the optics of the contact lens.

In every chapter theory, practice and interpretation are mixed in a pedagogical manner giving the maximum understanding.

11/8

Cool S. J. & Smith E. L. (Eds) Frontiers in visual science Springer Verlag Berlin 1979 p. 412 Price US Dollar 41.80

This volume comprises the proceedings of the University of Houston College of Optometry Dedication Symposium held March 1977 in Houston Texas USA. In the preface it is stated that the intention was "to cover the state of the art knowledge in all areas of visual science investigation." With this magnificent aim fulfilled by 70 papers it will immediately be understood that a review is not possible. There is an overwhelming amount of material but unfortunately no author or subject index.

With its very broad spectrum almost everyone may find something of interest. However, it is likely to find the whole text. The book should be screened by those engaged in research.

11/8

Allen F. W. Essentials of Ophthalmic Optics Oxford Medical Publications Oxford and London Press New York Toronto 1979 127 pages Price Eng pds 19.50 net

The book *Essentials of Ophthalmic Optics* is based on a series of lectures given by the author to ophthalmologists, nurses and orthoptists. Being a pocket size book of only 127 pages it is of course not intended as a comprehensive work on ophthalmic optics. However, the author has succeeded in explaining the essential principles in plain and competent language. References added after each section facilitate further studies by the reader. The book is divided into three sections. The first headed *Pure Optics* treats of the principles of light, refractive index, refraction, image formation, mirror and lenses etc. The second headed *Visual Optics* deals with vision, the eye as an optical instrument, objective and subjective refraction including retinoscopy among others. Included in this section are also passages treating of ophthalmoscopy and heterophoria. The third and final section is concerned with fitting of glasses and contact lenses, optical aids to partially sighted persons and the optical principles of the ophthalmological instruments.

The book thus covers a wide range of subjects and is recommendable to both ophthalmologists, orthoptists, nurses working within the field of ophthalmology and to students.

5/8

tung von Strukturen und Systemen in der Ophthalmologie R. Sachsenweger ed Nova Acta Leopoldina N F Nv 23o Band 50 Leipzig 1979 188 pages 8o illustrations 18 tables Price M 33 00

publication written in German records the information presented at the Leopoldina congress which took place in Halle (DDR) in the fall of 1977

the material is divided into 5 basic sections moderated by H Harms and H Sachsenweger the first section Systeme des Auges comprises information about the functional system development and biomorphogenesis of the eye The same chapter includes two interesting papers about retinal structures by H Sautter and about vitreous body structures normal findings and pathological changes presented by F J Reutsch

the second chapter the following articles are presented 1 Die Lokalisation von Störungen in den System des Sehorgans by H Aulhorn 2 Zur Struktur der oberen Hornhaut by E Winkelmann and 3 Strabismus concomitans – eine Störung des Bifixationsmechanismus by E S Awtussov These three detailed papers include very clear and didactical findings

the morphology and circulation of the anterior chamber fluids are presented as a 3rd chapter by W Rohen E Barany and H Goldmann

the next chapter diseases of the retina and choroid are considered The titles are Systemgebundene und Systemübergreifende angeborene retinale Störungen by Jaeger Makulaerkrankungen by H E Henkes and 3 Gefäßbedingte Erkrankungen der Netzhaut by H Goldmann

the final chapter P Niesel discusses the principles of therapy and rehabilitation on hereditary diseases of the eye The same section includes valuable reflections of E Barany on ocular therapy

Mackensen writes about Systemerhaltung und Wiederherstellung als therapeutisches Konzept aus der Sicht der Ophthalmochirurgen

finally an interesting and actual theme Vitrectomia via pars plana is presented by R. W. Phillips

the publication contains much valuable and detailed information and cannot be summarized briefly

there are useful references black and white illustrations as well as very instructive findings and tables

this book gives an excellent theoretical and practical information and will be of great interest to all ophthalmologists

Danuta Marushak

Handbuch Ophthalmologie A short textbook Georg Thieme Publishers Stuttgart 1979 Price DM 29 80

the translated edition has like the well known German edition advantages and drawbacks advantages are the small pocket book size the beautiful illustrations and the systematic presentation of the subject

the drawbacks are the many scientifically unfounded treatments e.g. vitamin A ointment for ophthalmic zoster steroid drops or ointment for optochiasmotic arachnoiditis or iodine application after abrasion and several misleading references to pages elsewhere in the book probably an error due to the translation

Niels Ehlers

William H Haremer Synopsis of Ophthalmology The C V Mosby Company
Toronto London 1979 675 pages 364 illustrations Price US Dollars 90.00

Synopsis of Ophthalmology was written for the purpose of informing general practitioners technique of examination diagnostics and therapy within the field of ophthalmology

In this book the individual subjects are described with a wealth of details. There is a chapter on the diagnostic significance of eye examination in relation to disease elsewhere in the body. A passage dealing with the anatomy and physiology of the eye is followed by comprehensive chapters on case recording and physical examination. The author stresses the great importance to ophthalmoscopy. Colour pictures of the fundus and numerous diagrams of ophthalmoscopic findings aid towards giving us an excellent survey and better understanding of the problems with which the examiner of the internal eye may meet.

The remaining chapters are concerned with ocular signs and symptoms, associated systemic diseases, neuro-ophthalmology, proper eye diseases and ocular trauma. Each chapter is furnished with many good illustrations.

The last chapter dealing with ocular pharmacology shows the author's special interest in this subject. He gives a careful account of the effects, advantages and disadvantages of various drugs.

The book ends up with a good index and an extremely useful list of words bearing ophthalmological terminology. This fact adds further to its value also as a reference book.

The book is highly recommendable also as a textbook for medical students.

S. F. 1980

Frank H. Newell Ophthalmology Principles and Concepts The C V Mosby Company
Louis Toronto London 1980 640 pages 477 ill Price UK Pounds 15.50

This is a book intended as an introduction to the subject for both students and those interested in ophthalmology.

In this fourth edition, like in the previous ones, the author has aimed mainly at providing basic knowledge with conversance with ocular manifestations and clinical pictures. Concentration on ocular details has been largely avoided out of regard to non-ophthalmologists.

The book comprises four main sections. The first one deals with the relevant anatomy, physiology, biochemistry and pharmacology.

The second section treats of the taking of case histories and with eye examination. This section is of particular instructive value to students.

The remaining sections are concerned with eye diseases and injuries as well as ocular signs and symptoms in relation to general illness. These sections are likewise well arranged and well written. Certain details may be criticized, however. Thus, for instance, the Schiotz tonometer is mentioned as the most common provocative test in simple glaucoma, a remark which hardly deserves. In fact, many workers regard the test as worthless.

This book is about the same size and get up as the previous editions, still having a few colour pictures. All the illustrations are black and white, which may be a disadvantage in some of the cases of the clinical pictures.

Reading of the book has been a pleasure to me, and I can recommend it to all those interested in an introduction into the subject.

K. 1981

Tumors of the ocular adnexa and orbit Ed. A. Hornblass. Mosby St. Louis, Toronto, London 1979. pp. 336, 608 ill. 3 in color. US Dollar 69.00

Text of this book in 30 chapters by 98 contributors is based on a conference at the Lenox Hospital in New York City in 1977. The first 17 chapters deal with tumors of the ocular adnexa and the remaining with orbital tumors.

Emphasis is placed on diagnosis and therapy and only sporadically on histopathology. All views on diagnosis and treatment are compiled including ultrasound and CT scanning in orbital diagnosis. As is customary in proceedings of symposia panel discussions in each section are printed. The therapeutic methods are thoroughly reviewed and often illustrated by sketches.

The black/white figures, however, are poor, often unsharp and often unillustrative, probably because they have been reproduced from color diapositives used during the symposium.

Many are reproductions from journals. The advantage of this book is the presence in one volume and the compilation of diagnosis and therapy of adnexal and orbital tumors. It is mainly for ophthalmologists, plastic surgeons and radiotherapists.

O. A. Jensen

Perkins and F. Clifford Rose: Optic Neuritis and its Differential Diagnosis. Oxford Medical Publications. Oxford, New York, Toronto. Oxford University Press, 1979. 99 pages. English. pds. 90.00 net in UK.

The authors present 179 cases of optic neuritis referred to the Royal Medical Ophthalmology in London over a 15 year period.

Investigative techniques are analysed and compared. Symptoms and treatment are dealt in separate chapters and the problems of differential diagnosis are reviewed extensively. Frequency with which optic neuritis progresses to multiple sclerosis is discussed and the relationship between optic neuritis and multiple sclerosis is analysed on the basis of the literature and the authors' extensive experience. One of several interesting conclusions is that multiple sclerosis following optic neuritis pursues a more benign course both in terms of severity and disability than that following other forms of presentation.

The book is devoted to demyelination of a tiny part of the central nervous system, but to ophthalmologists interested in multiple sclerosis and neuroophthalmologists it will be of interest.

A. resten W. ork

International Society for Contact Lens Research

Notice of 1st Scientific Meeting at Royal College of Surgeons London September 3rd 1980

All individuals who have an interest in research and wishing to attend or participate write as soon as possible to Montague Ruben Moorfields Contact Lens Department 1 Street London EC1 UK

Details regarding membership can also be requested at the same time if required.

The International Society for Orbital Disorders

organises the 4th International Symposium on Orbital Disorders on August 31st, Sept 1st and 2nd 1981 in the International Congresscentrum RAI in Amsterdam
Topics of the congress will be Immunology Radiotherapy Chemotherapy and Surgery of the Orbit

For further information one can write to the secretary general J H A Gijzen V University Eye Hospital Wilhelmina Gasthuis de Helmersstraat 104 1054 EC Amsterdam Holland

Israel International Symposium on Glaucoma

will take place in Tel Aviv (Israel) september 3-5 1980 Contact Secretariat P O B 16271 Tel Aviv

Annual Conference of the Glaucoma Service of the Wills Eye Hospital

will be held in the new Wills Eye Hospital June 6-7 1980

For further information please contact

Kenneth Benjamin M D c/o Glaucoma Service Wills Eye Hospital
1601 Spring Garden Street Philadelphia Pa 19130 (215) 978 6380

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SPECIFIC CHANGES IN THE FUNDUS TYPICAL FOR THE SJÖGREN LARSSON SYNDROME An ophthalmological study of 35 patients

BY

S. JAGELL¹, W. POLLAND² and O. SANDGREN²

All Swedish cases with the Sjogren Larsson syndrome (SLS) i.e. 35 patients were studied. Glistening dots of unknown nature were seen round the foveola. These foveal and parafoveal changes seem to be a cardinal sign of SLS together with congenital ichthyosis, spastic di/tetraplegia and mental deficiency.

Key word: Sjogren Larsson syndrome — macular changes

Sjogren Larsson syndrome (SLS) is a genetically determined syndrome with autosomal recessive inheritance characterized by the three cardinal signs: congenital ichthyosis, spastic di/tetraplegia and mental retardation (Sjogren & Larsson 1972; Theile 1974) (Fig. 1).

Several authors have noted ophthalmological changes in SLS. Sjogren & Larsson (1972) found degeneration of the macula in three of twelve patients. The fact that the most marked changes were found in two of the oldest patients made Sjogren & Larsson suspect a slowly progressive macular degeneration. However, another four patients in the same ages did not present any changes in their ocular fundi. Other authors have found macular changes in children with SLS. Vissian et al. (1968) found a multicoloured glittering fundus in a four months-old girl. Gilbert (1968) examined a 2½ year-old boy who had macular lesions in both eyes. Surrounding the macular lesions were small glistening dots. The electroretinogram was normal. Fluorescein fundus angiography revealed an increasing transmission of the choroidal vascular pattern. Theile (1974) summarized 111 reported patients with SLS. Different ophthalmologists examined 76 of these patients and they found macular changes in 38 patients and 15 of these showed glistening spots. Only

a few patients were examined by the same ophthalmologist. It is well known that patients with ichthyosis may have keratoconjunctivitis, blepharitis and corneal opacities (Cordes & Hogan 1939, Hammerstein et al 1958, Jonasson et al 1976, Sever et al 1968).

Specific macular changes combined with ichthyosis have not been reported. Observations of macular changes of different types in half of the SLS patients (Theile 1974) could be explained by the assumption that SLS depends on a systemic disturbance affecting the central nervous system as well as the skin. When one of us (S.J.) made an investigation of the SLS patients in Sweden, an identical type of fundus change in a varying degree in SLS patients was found, motivating further investigation.

The aim of the present study was to find out 1) if glaucoma is a pathognomonic change in the eyes of SLS patients, 2) the age of onset and whether 3) there is any correlation between macular changes and symptoms of a defective central nervous system.

Material and Methods

Ophthalmological examinations were performed on all patients with SLS (20 males and 15 females). The age range was from 1 to 71 years. The examination included ophthalmoscopy, slit lamp examination, electroretinogram in two cases and photography of the fundus. As a control group 14 patients with normal vision were examined as well as nine mentally retarded patients with normal vision and ten patients with mental retardation and spastic diplegia. None of the individuals had SLS.

Results

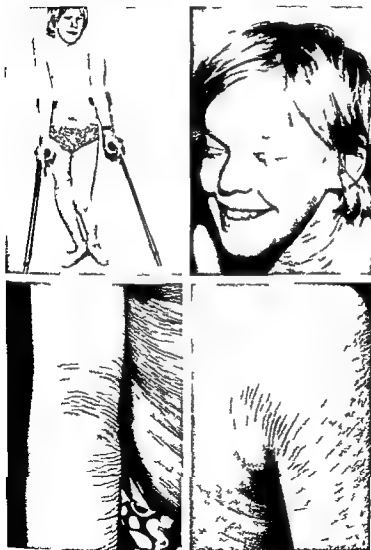
The major ophthalmologic findings of the 35 patients with SLS examined by the present authors are summarized in Table I. Numbers of patients are according to Jagell 1980. (There are altogether 58 SLS patients known in Sweden, numbered from 1 to 58. Only 35 patients were examined in this investigation; 23 patients are deceased.)

Visual acuity

Visual acuity was difficult to measure because of mental retardation. It was estimated in 12 patients to values in most cases between 0.2 to 0.5 with a mean about 0.4.

ve error

active errors were converted to spherical equivalents. Myopia was found in 10 patients of whom three patients had severe myopia with more than or equal to -6.00 D. Hyperopia was found only in one patient.



F = 1

Fig. 1. 15 years-old child with spastic diplegia, light mental retardation and generalized ichthyosis.

Table 1
Ocular findings in 35 patients with Sjögren Larsson syndrome

Patient number*	Age years	Vision	Refractive error Diopter	Ptosis	Blepharitis	Conjunctivitis	Punctate keratitis	Corneal opacities	Macular glistening dots
11	71	P/P		-	-	-	-	-	
17	68	P/O 1	+1°(op)	+	+	+	+	+	
21	58			-	+	+	+	+	
22	57	O 3 O 3	-1/-1	-	-	+	+	+	
26	51			-	-	+	+	+	
28	49			-	+	+	+	+	+
29	45			-	+	+	+	+	+
30	42	O 2 O 4		-	+	+	+	+	
31	40			-	+	+	+	+	
33	39			-	+	+	+	+	
34	39			+	+	+	+	+	
35	31	O 2 O 1	-2.2/-2.1	-	-	+	+	+	
36	29	O 4 O 5	-1.5/-1.1	-	-	+	+	+	
37	26	O 5 O 5	-0.2/±0	-	+	+	+	+	
38	22		+1.5/+1.5	-	-	+	+	+	
39	21			-	+	+	+	+	
40	21	O 2 O 3	-	-	+	+	+	+	
41	11		-3.2/-1.5	-	-	+	+	+	
42	10	O 4 O 3	-4/-4	-	-	+	+	+	
43	10		-1.5/-1.5	-	+	+	+	+	

Fig 2

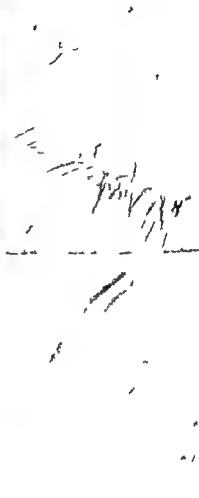


Fig 4

Fig 2 Pat No 40 Eye fundus photograph taken through Goldmann's three-mirror glass

Fig 3 Pat No 21 58 years-old with slight ichthyosis around the eye blepharitis vitis punctate keratitis and corneal opacities in the lower half of the cornea

Fig 4 Glistening dots in the fovea of pat No 51

Fig 5 Glistening dots in the fovea of pat No 30

al and conjunctival changes

A form of ectropion of the lower lids was found in two of the oldest patients. Keratitis and conjunctivitis with redness of the margins of the lids and hyperaemia of the conjunctiva were found in most of the patients (Fig. 3).

None of the patients had corneal changes with equal distribution in both eyes of varying severity from punctate epithelial erosions to grey stromal opacities with regularisation (Table I and Fig. 3). Stromal engorgement was seen more often in the older patients. The corneal changes were mostly situated in the lower half of the cornea (Fig. 3). The stromal opacities were always located in the anterior half of the cornea and were in most cases subepithelial.

Two patients (Nos. 11 and 17) showed dense cataract and lens extraction has been performed on one eye in one of these patients (No. 17). Another patient (No. 21) showed slight cataract in one eye. These three patients are the oldest in our material, 71, 63 and 58 years, respectively.

The fundus was thoroughly examined in 30 of the patients. The examination of the fundus was impossible in five of the patients because of cataract or corneal changes and poor and poor cooperation in one patient. All the 30 patients had an identical

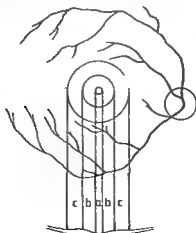


Fig. 6

The diagram shows the foveola (a), foveola (b) and parafoveal area (c) with some glistering dots of varying sizes.

type of retinal changes around the foveola in the fovea and parafoveal areas in Fig 2-4 and 5. These foveolar changes could be described as glistening dots. Most dots are seen in the fovea which often has somewhat more dots than those seen in the parafoveal area (These areas are seen in Fig 6 and according to Hogan 1971). The number of dots vary in different patients about 5 to 50 with about the same number in both eyes in each individual. They have an irregular shape and are of different size. Most often they are separated from each other but in some of the patients several dots seem to be near each other or confluent. One patient (No. 40) was examined with Goldmann three mirror contact glass. In this view the glistening dots looked like irregular bodies with unclear limitation in their periphery like low lying yellow white tops and greyish slopes situated in the superficial layers of the retina as seen in Fig 2. Nobody in the control group had similar macular changes. The amount of glistening dots does not seem to be related to age or the degree of mental retardation or spasticity.

Other eye findings

Almost all patients had marked photophobia. EOG performed in two patients (No. 30 and 51) were normal.

Discussion

Ophthalmological examination was difficult to perform because of mental retardation and spasticity which are both severe in most individuals with SLS. Furthermore, almost all patients had marked photophobia.

Most patients have a marked ichthyosis over almost the whole body but are so slightly involved that they are regarded to be free from any ichthyosis. Still some have an obvious and others a slight ichthyosis on the forehead. There is involvement of the orbital region.

We regard the blepharo-conjunctivitis and punctate keratitis with epithelial changes to be a symptom of the ectodermal disease SLS. Patients with SLS and ichthyosis have pronounced changes in the superficial part of the cornea. The photophobia is probably an effect of the punctate keratitis. We consider the corneal changes to be an effect of defective epithelium.

Senile lens changes were seen in three patients. There seem to be no association between cataract and SLS. There was an unusually high incidence of cataracts in our study. Those patients examined with regard to visual acuity had abnormal values. It is however difficult to measure the visual acuity in patients with mental retardation. The decreased visual acuity could be secondary to the mental retardation or secondary to their macular changes.

glistening dots in the macula have been reported in 15 of 76 patients according to Jagell (1974) as stated above. In our study we found glistening dots in all the SLS patients examined. Our investigation included nine of the 13 patients who were ophthalmologically examined in the study of Sjogren & Larsson (1957). Four of the patients including the three with macular degeneration are deceased. Of the remaining nine patients three patients have not SLS (Jagell 1980). Of the remaining patients with SLS we were able to examine the fundus of five who all had glistening dots. Sjogren & Larsson (1957) did not mention glistening dots in their

own opinion ■ that all patients with SLS have glistening dots in their fundi from childhood. The changes are probably stationary. The reason why other authors do not report glistening dots in all their SLS patients ■ probably that the changes often are so slight that they are easily overlooked or their significance has not been understood. The difficulty in examining the patients may also have played a part. We did not observe any correlation between the amount of glistening dots and the severity of spasticity, mental retardation, ichthyosis or age. Histological examination of glistening dots (snailtracks) in the peripheral fundus showed fatty degenerated microglia cells (Daicker 1971). We have not yet been able to make any histological examination of glistening dots in the fovea.

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NATURAL FAT IN EXTERNAL EYE

Vital Stained by Sudan III Powder

BY

M S NORN

Examination of 113 eyes vital stained by Sudan powder revealed that fat on the skin of outer canthus may pass onto the precorneal film (in 68%) and that the most reliable staining is obtained by application on the bulbar conjunctiva below the limbus corneae at 6 o'clock.

In 41% the precorneal film had stained drops or foam with a tendency towards an increase among pathological cases (infectious conjunctivitis and blepharitis). An increased amount of fat was found in the mucous thread of eyes affected with infectious conjunctivitis and lagophthalmos. In a small number of pathological cases fat was also detected on cornea, bulbar conjunctiva and inferior fornix.

The fatty layer on the lower lid margin was stained more frequently and more intensely than that on the superior.

The secretion of the meibomian glands was stained in no more than a scant one half of the glands (on an average 44%) independently of age, sex, site and diagnosis.

Key words: oil - lipid - fat - sebaceous - Meibomian glands - cornea - conjunctiva - vital staining

Natural fat on conjunctiva and cornea can be detected by Sudan vital staining and a water insoluble Sudan can be applied in ointment or oil and used as a vital staining (Norn 1963, 1977).

This only gives information about the fate of the ointment resp. oil but not concerning the natural occurring fat in the external eye. For this purpose we can use a powder as suggested by McDonald in 1969.

The purpose of this paper is 1) to work out an application method for Sudan staining of the external eye 2) to use the method on a clinical material.

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Sudan III powder was used (Merck 1380) The powder was introduced by means of a small wooden stick. Only relatively few granules were applied, thus giving no additional intensity of staining. Moreover, excess dye was removed from the surroundings with a consequent risk of staining hands and clothes. It will stand ordinary washing. The wooden stick was therefore discarded immediately after use.

The site of application was found to be critical. On application in the fornix the granules became enveloped in the mucous thread and transferred on to the skin of the inner canthus. In four out of 18 cases this therefore failed at the first attempt.

When applied on the skin of the outer canthus with closed eye the stained structures inside and upon the lid margin in 34% upon bulbar conjunctiva in 32% inside alone in 2% while neither lid margin nor eye was vital-stained.

The method preferred was that of applying dye granules on the conjunctiva just below the limbus corneae at 6 o'clock. This gave unperformed vital staining in 100% (45 cases). Evidently the granules were not covered by mucus until after they had been moved about by blinking and given off their components present in the external eye including the margins of both lids.

In the slit lamp (Haag Streit — magnification $\times 10-15$) it was possible by lighting (the light beam beside the granule) to distinguish between non-vital Sudan granules and proper vital staining of fat. The granules were black while the fat had a beautiful red colour. Direct lighting gave a red colour to both in the presence of fairly large amounts of fat we saw red drops that were smaller than the granules.

Proper vital staining was roughly graded from 1 to 5 within the different regions, 3 indicating moderate staining, 2 weak, 1 minimum, 4 fairly extensive, maximum. The values were read 10-20 min. after the staining.

Material

A total of 113 eyes were subjected to vital staining, 45 by application below the limbus at 6 o'clock, 50 on the skin of the outer canthus with closed eye and 18 elsewhere. Of these 17 had to be ruled out owing to inadequate staining, the remaining 96 constituted the clinical material comprising 49 normal and 47 diseased having the following diagnoses (numbers in parentheses): conjunctivitis (3), keratitis (6), corneal dystrophy (12), corneal abrasion (1), blepharitis or conjunctivitis (7), lagophthalmos (2), postoperative (9), miscellaneous (1).

Table I

external eye vital stained by Sudan III 49 normal eyes and 47 diseased (full text). The figures indicate the percentage stained in the region concerned (the figures in parentheses represent the mean staining grade)

	Pre corneal	Cornea	Bulbar conjunctiva	Caruncle	Inferior fornix	Mucous thread	Inferior margin	Superior margin
Normal	41	0	0	27	0	53	90	86
Mean grade	(0.6)	(0)	(0)	(0.4)	0	(0.8)	(2.7)	(2.3)
Pathological	60	6	17	21	4	81	100	87
Mean grade	(1.0)	(0.1)	(0.3)	(0.4)	(0.04)	(1.3)	(3.1)	(2.5)

Result

Fat was present in scant amounts only inside the lid margins of normal eyes stained by Sudan vital staining. Precorneally we saw fine drops or foam formation. In the caruncle a red ring was observed round a small number of ciliary roots indicating presence of fat in the hair follicle. Odd scattered fat drops occurred in the mucous thread of the inferior fornix (Table I). In the pathological cases corneal fat tended to be more frequent and present in larger amounts than in normal eyes (difference not significant) particularly so in cases of infectious conjunctivitis, blepharitis and after operation often occurring in lagoons. Fat adhered to the cornea was seen in cases with Dellen and corneal abrasion.

In some pathological cases (lagophthalmos round a Bitot's spot etc.) fat was adhered on the bulbar conjunctiva and in the inferior fornix. This was never seen in normal eyes. Stained fat was never observed on the plica semilunaris, the tarsus nor Marx line. The mucous thread of diseased eyes was more often than that of normal eyes seen to contain fat (81% against 53% $P < 0.02$ Student's *t* test). This was particularly so in cases of infectious conjunctivitis and lagophthalmos.

The lid margin naturally presented intense diffuse staining of the fatty layer sharply demarcated from the lacrimal river (= tear prism) and thus also from the Marx line. Vital staining with Sudan and lissamine green showed this beautifully. A broad band of stained fat was formed within which a green Marx line was seen where the two dyes adjoined without ever mixing (Fig. 1).

The boundary line was noticed to be irregular in several cases unlike the conditions in rabbits where the line is regular festoon shaped.

Fat was observed more frequently and in larger amounts on the lower lid margin than on the upper (Table I $P < 0.05$). The location depended on the method of



Fig 1

Natural fat stained by Sudan powder and Marx line stained by lissamine green. The outlets of the Meibomian glands are stained at the lateral part of the eye lid margin.

application. The staining was most often located laterally when the dye was applied on the skin of the outer canthus (92%) and most often medially on application below the corneal limbus at 6 o'clock (87%). However external application was medially (8%) while in 9% application at 6 o'clock stained the margin laterally.

Table II

Sudan vital staining of secretion at the outlets of Meibomian glands in percent of the number of possible glands. An average of 41% were stained in the individual subject.

	Upper lid	Lower lid	Number of glands	Number of eyes
Normal	31	46	489	23
Pathological	45	51	601	28

outlets of the Meibomian glands were seen at the line where the fat stained the adjoined Marx line. The row of glands may be so irregular that some open thin Marx line. As might be expected, the fat present at the outlet was in 1 case stained intensely red. However, in 56% of the glands the outlets had red unstained even though the environments were stained. Evidently the was in such cases covered by a fat free membrane. Staining of the outlet of Meibomian gland was independent of age and sex. No significant difference was between upper and lower lids nor between normal and diseased eyes (Table II). A non stained Meibomian gland may become stained after expression. A stained gland may burst and send a cloud of fat into the precorneal film. A Meibomian gland in the aqueous phase (Marx line) may show prominence of fat while at the same time the neighbouring gland in the lipid phase may not be stained by Sudan.

Discussion

It has been shown previously that ointment applied on the skin of the outer canthus of the eye is closed may be transferred to the precorneal film by blinking and be taken up by the mucous thread in the inferior fornix. It will then pass with this on the skin of the inner canthus (Norn 1977).

Using Sudan powder it was shown in the present study that natural sebum from the skin may pass the same route. This affords evidence to suggest that the superficial fatty layer of the precorneal film consists of sebum from the skin plus fat from the Meibomian glands. The latter are presumed to be secretorily active at all times, although only more than half of them being covered by a membrane.

Previously subjected mucous threads removed from the inferior fornix were examined microscopically for fat (Norn 1963). As also noticed in the present study, the amount seemed to have increased in cases of lagophthalmos. The evenness of the precorneal film is so thin that it will not be detectable by Sudan staining. The layer can, however, be measured semi-quantitatively by interference contrast in the slit lamp (Norn 1979). It is about 100 nm thick in normal eyes – thicker in dry eye, pharyngitis and infectious conjunctivitis. This is in agreement with the results of the present investigation.

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CLINICAL ASSESSMENT OF COLOUR DISCRIMINATION IN SENILE MACULAR DEGENERATION

BY

K. J. BOWMAN

The colour discrimination of 15 subjects manifesting senile macular degeneration was investigated over a wide range of illuminances using the Farnsworth Munsell 100-Hue test and Panel D 15. Ten subjects of similar ages with normal colour vision were investigated concurrently to provide a control group. Colour discrimination was shown to deteriorate with decreasing illuminance this being more marked for the subjects with senile macular degeneration than for the normal subjects. It is demonstrated that the FM100 is the preferred test for assessment of colour discrimination loss in senile macular degeneration with early visual acuity loss. The Panel D-15 is more useful as acuity loss becomes more marked.

Key words: senile macular degeneration - colour vision - eye disease - age - colour discrimination

course of senile macular degeneration (SMD) can be monitored by the assessment and measurement of a number of visual functions. Visual acuity and its discrimination are obvious and simple functions to assess. Central visual fields can be plotted but the procedures are less simple if meaningful results are to be obtained.

While visual acuity is the traditional and conventional means of assessing visual function, monitoring colour vision can be a useful addition tool in the manage-

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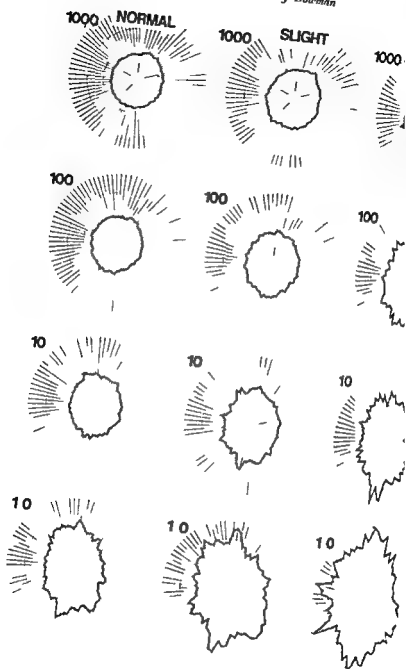


Fig. 1
Group mean FM100 patterns for the normal, slight and defective vision at four illuminance levels (1000, 100, 10 and 1.0 lux)

of senile macular degeneration (Perdriel et al 1970). This may be particularly the early stages of the disease process where acuity changes are absent or small or where the rate of acuity deterioration is slow and difficult to assess accurately.

Farnsworth Munsell 100-Hue test (Farnsworth 1943, 1957) is admirably suited to the clinical assessment of colour discrimination although when functional loss is gross and colour discrimination poor meaningful results are more difficult to obtain (Chisholm 1969). Recent advances in the automation of the test and plotting of the FMI100 results (Taylor 1978, Donaldson et al 1978) should provide an impetus for its more widespread use in larger clinics whereas its use has generally been restricted to research departments.

• dyschromatopsia accompanying senile macular degeneration is generally categorized as a blue-yellow or tritan defect (Cox 1961, Francois & Verriest 1961) although non-specific defects have been reported (Campbell & Ruttler 1972, Davies 1972). These investigations have usually been conducted at various levels of luminance and age-matched control groups have not been studied concurrently. The two factors are important since FMI100 performance in particular has been demonstrated to be both age and illuminance dependent (Verriest et al 1962, West et al 1963, Cornu & Harlay 1969).

The present study examines the colour discrimination of subjects manifesting senile macular degeneration, the effect of illuminance on discrimination and compares their performance with that of an age-matched normal control group.

Materials and Methods

Seventeen subjects (mean age 66.5 years) with senile macular degeneration and an age-matched control group of 10 subjects (mean age 64.0 years) with normal colour vision and manifesting no ocular pathology were studied. Subjects with media opacities were excluded from participation in order to equalise selective absorption of light between groups and also to ensure visual acuity changes were due solely to senile macular degeneration. The 15 subjects with senile macular degeneration were divided into two groups designated slight (8 subjects) and definite (7 subjects) solely according to visual acuity measurements using the acuity chart of Bailey & Love (1976). The criteria for classification were Slight $6/b - 6/12$ and Definite $< 6/12 - 6/30$ each group representing four acuity lines on this chart, a difference of 0.4 log units of minimum angle of resolution. Following a familiarization trial at 1000 lux to reduce learning effects, the FMI100 was performed twice by each subject in a counterbalanced ABBA sequence at each

the most consistent tritan patterns (Fig. 5) whereas these traces appear at levels of illuminance for the slight group. Since the Panel D-15 is administered at levels of at least 100 lux it is evident that the detection of defect in SMD by this test is doubtful until acuity is reduced below approximately 6/12. Until this reduction in acuity is reached the FM100 is the preferred method to detect the loss of colour discrimination. This relationship between visual acuity and Panel D-15 performance in senile macular degeneration has previously been reported by Helme & Krause (1972).

Discussion

Older subjects with normal colour vision suffer marked loss of dark surface colours in conditions of low ambient illuminance. Subjects with senile macular degeneration suffer more marked loss of discrimination related to the severity of the acuity loss.

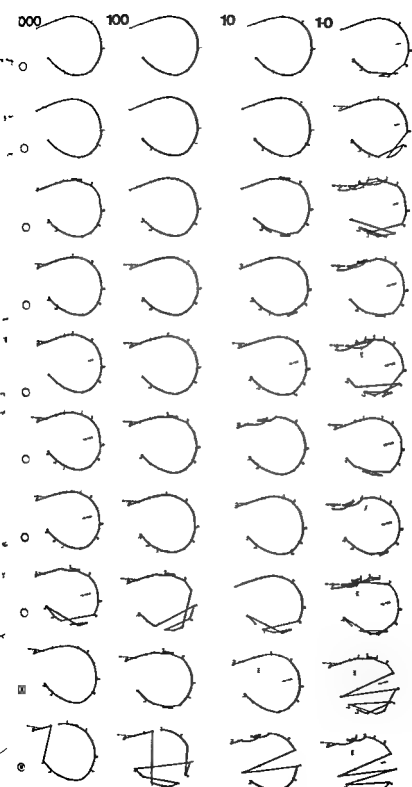
The FM100 would appear most useful for the clinical assessment of colour discrimination with early visual acuity loss. As this acuity loss becomes substantial and FM100 results less definitive the Panel D-15 provides a more reliable technique.

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Fig. 3

Panel D-15 patterns for the ten normal control subjects at each of the four illuminance levels.



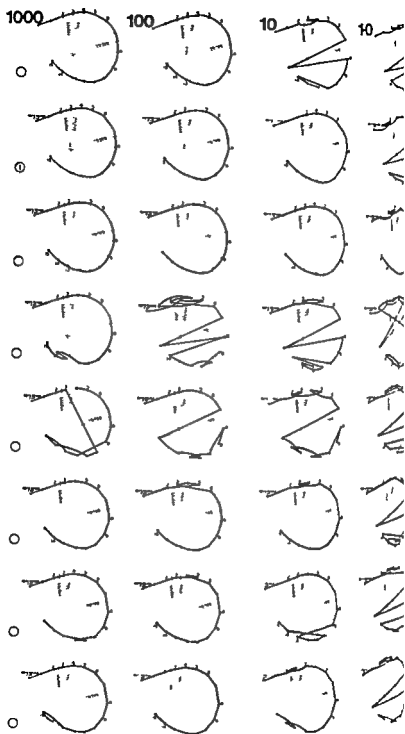


Fig. 4

Panel D 15 patterns for the eight slight EMD group subjects at each of the four levels

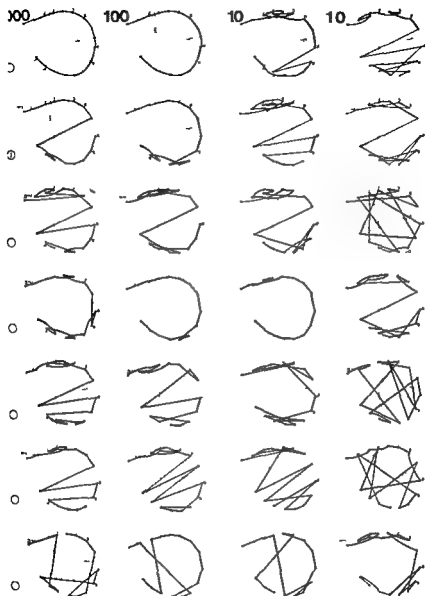


Fig 5

perceived D-15 patterns for the seven definite SMD group subjects at each of the four illuminance levels

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FLUORESCEIN ANGIOGRAPHY IN DIABETIC CHILDREN

BY

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In 11 diabetic children aged 10–14 years with normal ophthalmoscopy fluorescein angiography revealed slight diabetic retinopathy in maximally three aged 12–14 years. These findings are discussed in relation to the controversial results published in a few other fluorescein angiographic studies on diabetic children.

It is concluded that normal ophthalmoscopy does not exclude the presence of diabetic retinopathy and that fluorescein angiography is a valuable method in the early diagnosis of diabetic retinopathy.

Key words: fluorescein angiography – diabetic retinopathy – diabetic children – diabetes mellitus

Until about 1960 the development of ocular fundus changes could be observed and followed by ophthalmoscopy and fundus photography only. Since the introduction of fluorescein angiography by Novotny & Alvis (1961) more information has been obtained on the retinal circulation and the development of pathologic fundus changes in various diseases among others in diabetes.

Until this study started only a few fluorescein angiographic studies had been published on fundus changes in juvenile diabetes.

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Barta et al (1972) and Brooser et al (1975) found a remarkable incidence of retinopathy in diabetics under the age of 15 years.

Toussaint & Dorchy (1974) in their study of juvenile diabetics did not see retinopathy before the age of 15 years.

The results of Barta et al (1972) and Brooser et al (1975) are in striking contrast to the general concept that diabetic retinopathy seldom occurs before age 15. Therefore we felt that further fluorescein angiographic investigations were required and started a fluorescein angiographic study on diabetic children in 1977.

Material and Methods

The study included 63 diabetic children aged from 10 to 14 years. Thirty-two girls and 31 were boys. All were randomly selected among outpatients from the Department of Paediatrics, Glostrup Hospital and the Steno Memorial Hospital, Gentofte. The investigations were performed after obtaining informed consent from both the children and their parents.

Twenty-seven of the children had a family history of diabetes. Two of the children were siblings but not twins.

The ages of the children at the time of the ophthalmologic examination ranged from 10 to 14 years with an average of 12 years. The duration of diabetes ranged from one month to 13 years, average 4.6 years.

All patients were treated with diet and highly purified porcine insulin, and 65% of them received insulin twice a day. The dose of insulin ranged from 0.5 to 1.5 IU/kg (average 0.74 IU/kg).

All patients were controlled in the diabetic clinics at regular intervals of 1 to 3 months.

During the observation period, which in most instances was from the time of diagnosis of the diabetes, 12 of the children had been hospitalized. Seven for ketoacidosis, 3 once for diabetic coma, and 2 twice and nine times respectively for severe hypoglycaemia.

Body weight and height were compared with ideal body weight and height from Danish normal material (Andersen et al 1974) and showed on the average normal values.

Glycosuria was measured in fractionated urine collections almost daily at home and in 24-hour urine at the control visits.

Status of regulation (Table I) was grouped into 3 categories: good, moderate and poor. The grouping was based on accurate notes in the records on the number of admissions necessary for regulation, including episodes of ketoacidosis.

Table I
Estimated status of regulation

Regulation group	Number of patients	
	Total (n = 63)	With retinopathy
Good	27	2
Moderate	18	1
Poor	18	0

na or hypoglycaemia and the values of blood glucose, glycosuria, ketonuria and weight during the observation period.

None of the children had clinically overt late diabetic complications or presented other general diseases and none of the children received any other drugs than insulin.

The ophthalmologic examinations included refraction and corrected visual acuity, slit lamp examination, ophthalmoscopy, fundus photography and fluorescein angiography. The fluorescein angiograms were evaluated independently by two ophthalmologists.

The photographic records were made following maximal dilation using tropicamide (Mydracyl®) 1% and cyclopentolate (Cyclogyl®) 1%.

Colour fundus photographs were taken with the Topcon fundus camera on Kodachrome 25.

Retinal fluorescein angiography was performed on the posterior pole of the right eye in each child as the early sequence study area. In the initial 10–120 seconds after photographs covering an area of at least 30° around the disc were taken.

Table II
Diabetic children with abnormal fluorescein angiography

Age (in years)	Sex	Diabetes in the family	Duration of diabetes (in years)	Number of microaneurysms
14	male	+	13	1
19	male	—	7	1
14	male		6	Several

following the main series. Additional photographs of the posterior pole were taken 10 min and 30 min after the injection of fluorescein. The dose of fluorescein (in a 10% solution) was based on body weight at about 10 mg/kg injected as a bolus into a cubital vein. The angiograms were taken with the fundus camera equipped with standard filters. No complications were observed in relation to the fluorescein angiographies apart from slight nausea in four cases.

Results

In all children ophthalmoscopy and fundus photography were normal. None of the children showed pathologic lens changes.

The fluorescein angiograms were normal in 60 cases. Three cases showed abnormalities (Table II). Two had changes which were interpreted as microaneurysms and one had some microaneurysms and dilated leakage of capillaries (Fig. 1). None showed alterations in the macular pigmentation.

All of the three children with retinal changes were considered to be in the state of regulation although one showed retardation of growth.

Discussion

The incidence of diabetic retinopathy rises slowly during the first five years of diabetes and more slowly in the younger age group than in the older age groups. In juvenile diabetes the general concept based on ophthalmoscopy has been that retinopathy rarely occurs before the age of 16 years no matter how long the diabetes has been present (Larsen 1960).

As fluorescein angiography often reveals more microaneurysms than can be seen ophthalmoscopically (Scott et al. 1963) this general concept might have changed somewhat.

In a fluorescein angiographic study Barta et al. (1972) proved a very remarkable incidence of microaneurysms in juvenile diabetics.

The results were based on a material of 111 diabetics, 49 boys and 62 girls, whom diabetes was diagnosed before the age of 14 years. All patients showed normal ophthalmoscopy but 61 (55%) presented microaneurysms on fluorescein angiography. Eighteen out of 40 (45%) presenting microaneurysms were 14 years old and 43 out of 71 (61%) were 15 years old or more. The incidence

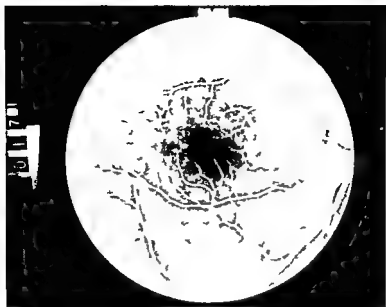


Fig 1

in patient No 3 Fluorescein angiogram (17 seconds after injection of dye) showing some microaneurysms and dilated leaking retinal capillaries temporal and above the fovea centralis

diabetic retinopathy rose not only in relation to the age of the child but also in relation to the duration of diabetes while no significant correlation was found between the control of diabetes and the occurrence of retinopathy

In another fluorescein angiographic study on 161 diabetic children with normal ophthalmoscopy Brooser et al (1975) found microaneurysms in 42% in the age group 3-10 years 59% in the age group 11-15 years and 86% in the age group 16-18 years With a duration of diabetes from 0-5 years 50% showed diabetic retinopathy with a duration from 6-10 years the incidence rose to 84% and in cases with a duration from 11-18 years the incidence was 74%

Toussaint & Dorchy (1974) and Toussaint et al (1976) examined 30 diabetics aged 9-32 years Diabetes was diagnosed before the age of 15 years Fluorescein angiography revealed no retinopathy in the age group under 15 years

In another fluorescein angiographic study on 87 diabetics with an onset of diabetes before the age of 14 years Dorchy et al (1977) found five cases (11%) of retinopathy among 45 children under 15 years of age They also found that the duration of diabetes and a poor control augmented the incidence of retinopathy considerably

Malone et al (1977) published a fluorescent angiographic study on 14 children aged from 5–18 years. Diabetic retinopathy was demonstrated by fluorescein angiography in 22 of which 12 were aged 8 to 15 years. Fluorescein angiography revealed vascular defects in 74% of the children who showed no abnormalities in the retinal colour photographs.

In the present material evidence of diabetic retinopathy was found in three (5%) out of 63 children. At present variations in the normal fluorescein angiogram have not been investigated. It is therefore not known whether microaneurysms can be present in normals. These three had had diabetes for 12–13 years (14% of the patients with a duration of diabetes more than 10 years). They were 12–14 years old and were considered to be in reasonable metabolic regulation although one showed retardation of growth. However they differ from the rest of the material.

These findings are in agreement with the findings of Toussaint et al (1971) and Dorchy et al (1977) but in striking contrast to the findings of Barta (1972), Brooser et al (1975) and Malone et al (1977).

The mechanism of development of diabetic microangiopathy and retinopathy is still not fully understood although much research has been done and several hypotheses have been put forward. Besides basic factors such as hyperglycaemia, metabolic changes in the tissues, the status of regulation, diet, insulin, and genetics have been postulated as factors influencing the development of diabetic retinopathy.

Can differences in these parameters explain the discrepancy in the results obtained?

Barta & Molnar (1970) found a definite tendency to retinopathy in young diabetics when retardation in growth was present. In our material however only one of the three presenting retinopathy on fluorescein angiography had retardation in growth.

Barta et al (1972) and Malone et al (1977) did not find any correlation between the degree of control and the occurrence of diabetic retinopathy while Dorchy et al (1977) found such a close relationship. However the degree of control or regulation are not adequately specified.

Differences in diet or insulin could be factors which might influence the development of retinopathy. Job et al (1978) split up the daily insulin requirement into two equal doses and showed that this regimen was effective in improving the diabetes regulation and delaying retinal changes. Specified information on diet, purification and concentration of insulin are however lacking in most materials.

Barta et al (1972), Brooser et al (1975) and Malone et al (1977) found a considerable number of children with retinopathy before the age of 10 years. In the present material none of those presenting retinopathy were pubescent.

independent influence on the development of retinopathy independent of the metabolic changes in diabetes was suggested by Brooser et al (1972) because of the frequent occurrence of microaneurysms soon after the onset of diabetes. The results of the present investigation, however, cannot support this theory since retinopathy did not occur in any case before diabetes had been present at least 5 years.

Some of the discrepancies in results obtained from these fluorescence angiographic studies may be due not only to differences in the materials etc. but also to a different interpretation of the fluorescence angiograms. Attention should be drawn to the fact that defects in the pigment epithelium can be impossible to distinguish from microaneurysms unless a comprehensive sequence of photographs is available and carefully studied.

It can, however, be concluded that normal ophthalmoscopy does not exclude the presence of diabetic retinopathy and that fluorescence angiography is a valuable aid in the early diagnosis of diabetic retinopathy.

A longitudinal study will be followed up on the same material at two-year intervals to show the natural history of the disease and to ensure the correctness of the interpretation of our results.

Acknowledgments

The authors wish to express their gratitude to Kirsten Dyrlov, Department of Paediatrics, Copenhagen Hospital, for outstanding technical assistance.

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FLUORESCEIN ANGIOGRAPHY IN DIABETIC CHILDREN

a Follow up

BY

KIM FROST LARSEN and KIRSTEN STARUP

The present study is a fluorescein angiographic follow up of 60 diabetic children (aged 12–16 years).

At the follow up with two-thirds of the children in pubescence fluorescein angiography revealed diabetic retinopathy in 10 (17%) of the children.

The results indicate puberty as a probable determining factor influencing the onset of the diabetic retinopathy.

Key words: fluorescein angiography – diabetic retinopathy – diabetic children – diabetes mellitus

means of fluorescein angiography microvascular changes usually can be demonstrated in diabetes mellitus before they are ophthalmoscopically visible and even in cases with ophthalmoscopically visible microaneurysms fluorescein angiography usually shows a larger number of microaneurysms than can be seen ophthalmoscopically. Fluorescein angiography therefore provides a more reliable method of detecting the initial signs of diabetic retinopathy and assessing the valence of this condition among diabetic children. Until the present fluorescein angiographic study was started in 1975 such studies on fundus changes in juvenile

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diabetics as had been published had given only few and rather conflicting results (Barta et al 1972 Brooser et al 1973 Toussaint & Dorch 1974).

These obviously conflicting results did not seem to be due to differences in the criteria for selecting the patients or the metabolic state. The present angiographic investigation designed as a long term study was started with a view of contributing to a clarification of the problem of the debut and pre-diabetic retinopathy in childhood and the possible influence of puberty on onset and development of the disease. The results of the initial investigation have been presented in an earlier article (Starup et al 1980). This paper presents results of the first follow up carried out in the autumn of 1977.

Material and Methods

The initial study included 63 children with insulin-dependent diabetes mellitus. Three of these were lost to follow up: one had moved abroad, one had refused examination on religious grounds and one had died from leukaemia. At the follow up girls and boys were equally represented. The ages of the children and the duration of IDDM at the two examinations and the follow up are shown in Table 1. In the period between examinations the percentage of children receiving insulin twice a day had increased from 60% to 87% and the daily dose of insulin had increased from an average of 0.6 (range 0.20–1.30 IU/kg) to 0.96 IU/kg (range 0.30–1.60 IU/kg) (Table 2). In the performance of the initial study two years ago two children had been hospitalized for episodes of severe hypoglycaemia but none of the patients had had ketonuria. The degree of long term metabolic control was based on body weight and linear growth of the children and showed on average normal values. None of the children had clinically overt complications or presented general diseases and none received any drug other than insulin. At the preliminary examination none of the children showed signs of puberty. At follow up 65% had grown into puberty. The study was performed after obtaining the consent of both the children and the parents.

Table 1
Age and duration of diabetes at the two examinations.

Examination	Number of cases	Age (in years) $\bar{x} \pm s.d.$	Duration of diabetes (in years) $\bar{x} \pm s.d.$
Preliminary	63	12 \pm 0.9	4.6 \pm 0.1
Follow up two years later	60	14 \pm 0.2	6.6 \pm 0.1

Table II
Dose and administration of insulin at the two
examinations

Examination	Daily dose of insulin (in IU/kg) $\bar{x} \pm \text{SEM}$	Percentage of children receiving insulin twice a day
Preliminary	0.74 \pm 0.08	67%
Follow up two years later	0.96 \pm 0.09	87%

ophthalmological examinations included refraction and corrected visual acuity slit examination, ophthalmoscopy, fundus photography, fluorescein angiography and perimetry. The technique was the same as in the initial study, but fluorescein angiograms in the present study were taken with a Kowa wide angle (42°) fundus camera, model RC W, equipped with Kodak W 15 and W 56 interference filters.

None of the children had mild and transient nausea immediately after the injection of fluorescein. Apart from this, no side effects were recorded. All angiograms from each patient were assessed independently by both authors, and the readings were further confirmed by an independent observer (Professor Hans-Walther Larsen). To rule out artifacts, a lesion had to be present at the same site in at least two photographs.

For perimetry, a simple test for visual dark adaptation was recorded by means of the Goldmann field perimeter (Registrier-Perimeter) made by Carl Zeiss (Jena, DDR). Perimetry was included in this study because of the probable predictive and prognostic value of this test in diabetic retinopathy (Frost, Larsen et al., 1990).

The exact test was utilized in the statistical analysis of the data.

Results

As reported in the initial study, maximally three of the children presented diabetic retinopathy.

In the present study, five of the children showed vascular abnormalities as single or few red dots on ophthalmoscopy. These fundus changes could all be seen on colour retinal photographs, and in addition, colour fundus photographs showed red dots in a further three children. In six of these eight children, the fundus changes were bilateral, while the remaining two showed single dots in the right eye only. None showed any hard exudates or alterations in the macular pigmentation.

Table III
Age and duration of diabetes at the follow up

Diagnostic group	Number	Age (in years) $\bar{x} \pm \text{SEM}$	Duration of diabetes (in years) $\bar{x} \pm \text{SEM}$
Total material	60	14.2 \pm 0.2	6.6 \pm 0.1
Retinopathy	10	15.2 \pm 0.1	7.6 \pm 1.5
No retinopathy	50	14.3 \pm 0.2	6.4 \pm 0.1

The fluorescein angiography revealed diabetic retinopathy in 10 children 17% of the total material including all of the eight children in whom retinopathy was demonstrated on the colour retinal photographs. The remaining 10 children shown normal fundi on both ophthalmoscopes and colour fundus photographs. It was obvious that the fluorescein angiograms demonstrated a greater degree of pathological fundus changes than could be noted on the colour photographs. Of the children had more than six microaneurysms in each eye and 4 had leaking retinal capillaries. None showed non perfused areas or neovascularization.

The children with diabetic retinopathy were older and had a longer duration of diabetes than the children without retinopathy (Table III) but these differences were not statistically significant.

Ninety per cent of the children with retinopathy had grown into puberty, only 62% of the children without retinopathy were in puberty and this difference was statistically significant ($P < 0.05$ (Table IV)).

The average daily dose of insulin was slightly but not statistically significantly higher among the children with diabetic retinopathy compared to the children without retinopathy. Furthermore in all children showing retinopathy at least

Table IV
Distribution of cases with retinopathy in relation to puberty

Diagnostic group	Pubescent	Non pubescent
Retinopathy	90%	10%
No retinopathy	62%	38%

Table V
Dose and administration of insulin at the follow up

Examination	Daily dose of insulin (in IU/kg) $\bar{x} \pm \text{SEM}$	Percentage of children receiving insulin twice a day
Total material	0.96 ± 0.09	87%
Retinopathy	1.05 ± 0.12	100%
No retinopathy	0.91 ± 0.09	81%

insulin was necessary in two daily doses while some of the children without retinopathy received insulin only once a day (Table V)

Two of the children presenting diabetic retinopathy had evidence of growth retardation as an indicator of poor diabetic control while the remaining eight children did not differ from the rest of the material in this respect. Only three of the children presenting diabetic retinopathy had a relative with diabetes. In the course of the study at two years the incidence of diabetic retinopathy in the children under study had increased from 5% to 17%.

In all 60 children nyctometry showed normal values. The cooperation between the investigators and patients was excellent throughout the study.

Discussion

The present discussion concerning the discrepant results of previous fluorescein angiographic studies in diabetic children has been presented in the initial study (Petäli 1980).

The results of our follow up study demonstrate a significant increase in the incidence of retinopathy in this study group in the course of about two years. In the children who had diabetic retinopathy at the preliminary investigation retinal microaneurysms were still present but at different sites in the retina. Our results demonstrate a statistically significant difference ($P < 0.05$) in the occurrence of diabetic retinopathy between the groups of pubescent and non pubescent children. On the other hand, our results show no statistically significant differences between the two groups with regard to age, duration of diabetes, dose or administration of

Table I
Age and duration of diabetes in the following

Diabetes Group	Number	Age	Duration
		(in years) $\bar{x} \pm \text{SEM}$	(in years) $\bar{x} \pm \text{SEM}$
Insulin-treated	51	34.2 ± 0.2	6.6 ± 0.4
Non-insulin-treated	10	35.2 ± 0.1	7.1 ± 0.1
Non-diabetic	51	34.3 ± 0.2	6.4 ± 0.4

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RESULTS OF PHOTOCOAGULATION IN DIABETIC RETINOPATHY AFTER LONG-TERM FOLLOW UP

BY

BIRGITTA ZETTERSTRÖM

Seventy three diabetics with retinopathy in whom one eye was treated by photocoagulation and the other was left untreated were followed up for 9 to 11 years. Visual acuity, visual field, dark adaptation curves and intraocular pressure were recorded. Biomicroscopy and photography of the fundus were carried out. Severe visual loss (< 0.1) was noted in 4% of the treated and in 9% of the untreated eyes. Minor subjectively unnoticed scotomas were found at visual field examination in 18 of 40 treated eyes. Dark adaptation curves showed abnormal values both in the treated and in the untreated eyes without any recordable difference. No contra indicating long term complications of the photocoagulation treatment could be demonstrated.

Key words: diabetic retinopathy - photocoagulation - visual field - dark adaptation

Photocoagulation treatment of diabetic retinopathy has been found to have beneficial effects according to the results of extensive well-controlled multicentre trials (Diabetic Retinopathy Study Research Group 1976, British Multicentre Photocoagulation Trial 1977, Hercules et al. 1977). The risk of severe visual loss as reduced and no contra indicating complications of the treatment have so far been noted. Meyer-Schwickerath, who was the first to introduce the photocoagulation method, discussed at an early stage the cause of the favourable effect in diabetic

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retinopathy. As early as 1969 he suggested that the explanation might be the result of scarring of some parts of the retina the metabolic conditions in the remaining untreated parts could have become more favourable. This hypothesis has in part been accepted and developed. The purpose of the present study was to study the long term effect and any late complications of photocoagulation.

Material and Methods

Over the period 1968-1970 156 patients were treated by photocoagulation. Of them were re-examined in 1979 that is 9 to 11 years after treatment. Eighty three of the original 156 patients could not be traced for various reasons: they had died (18) were too severely ill to be examined (9) moved (23) or did not want to attend the follow-up examination (3).

At photocoagulation in 1968-1970 only one eye was treated and the other was used as a control. At the start of the treatment period 38 of the 156 patients had proliferative retinopathy without growth of vessels in the vitreous cavity and without connective tissue. 15 had moderate to severe simple retinopathy that is absence of neovascularisation but more or less profuse macular haemorrhages and exudates. The retinopathy was of similar appearance in the treated and the untreated eye. The difference in visual acuity between the treated and the untreated eye was maximally 0.2. The eye with the lowest visual acuity was selected for treatment. At the start of the treatment period visual acuity was 0.5-1.0 in 70 and 0.4-0.1 in 3 of the 73 followed-up patients.

The apparatus used was Zeiss Oberkochen photocoagulator. Appennings and 4.5 were used and a total of 200 to 500 lesions were produced in each eye on two or three occasions.

At the follow-up examination the patients were asked about any eye trouble for the purpose of assessing any subjectively observed visual field defects or any reduced dark adaptation. Visual acuity, media and fundus were checked and recorded photographically in all the eyes. In one treated and one untreated eye the fundus could not be photographically recorded due to opaque media. Visual field was examined in 40 of the patients. Dark adaptation curves on Wecker's adaptometer were recorded for the treated and the untreated eye separately in 10 of the 73 patients. The ages of these 10 patients ranged from 46 to 60 years and visual acuity in both the treated and untreated eye was 0.1-0.4. Intraocular pressure was measured in all the eyes by applanation tonometry.

Table I

Visual acuity of photocoagulation treated eyes and untreated fellow eyes before and after follow up for 9 to 11 years

Visual acuity	0.5-1.0	0.1-0.4	< 0.1
<i>Treated Eyes</i>			
1968-70	70 (96%)	3 (4%)	0 (0%)
1979	57 (78%)	13 (18%)	3 (4%)
<i>Untreated fellow eyes</i>			
1968-70	70 (96%)	3 (4%)	0 (0%)
1979	39 (44%)	23 (31%)	18 (25%)

Results

Visual acuity of the treated and the untreated eyes at the start of treatment in 1968-1970 and after follow up for 9 to 11 years is recorded in Table I. It will be seen that the treated eyes showed a percentage impairment of visual acuity but that severe impairment (< 0.1) occurred in only three eyes after 9 to 11 years. At the check up in 1979 two of the latter three patients had also visual acuity < 0.1 in the untreated eye and one had visual acuity of 0.3 in the untreated eye which means that only one patient had severe visual loss in the treated eye alone. A larger proportion of the untreated eyes showed impairment of visual acuity and 18 eyes had severe visual loss (< 0.1) (By χ^2 test according to MC Nemar $P = 0.0001$).

Disjunct retinal atrophy with very narrow vessels and some optic atrophy were noted in 28 of the treated but in only five of the untreated eyes ($P \approx 0$). Careful microscopy at the follow up in 1979 revealed that neovascularisation was not present in 17 of the 58 eyes with proliferative retinopathy treated in 1968-1970. In untreated fellow eyes neovascularisation of varying degree was noted at the follow up in all the eyes in which the fundus could be inspected. In eight of the treated eyes the condition of the fundus could not be assessed because of vitreous haemorrhage or cataract. Of the 15 eyes with background retinopathy alone treated in 1968-1970 two had developed proliferative vascular changes at the follow up. Of the 15 untreated fellow eyes 11 showed proliferative retinopathy at the follow up examination ($P = 0.002$).

The photocoagulation scars were highly atrophic mostly with intensive pigmentation. Photographic studies showed that the scars had also increased in size compared with their appearance at one to three years after the photocoagulation.

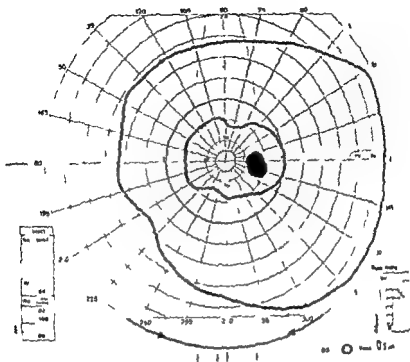


Fig 1

Visual field showing minor scotomas post photocoagulation treatment

Visual field tests using Goldman's perimeter with two isopters objective size I intensity of 4 and objective size I intensity II and screening of the blind spot in treated eyes revealed minor scotomas in 18 in which no symptoms had been reported subjectively. An example of a visual field from one of the patients having minor scotomas is demonstrated in Fig 1.

Adaptation curves for 10 patients are shown in Fig 2 for the treated eyes and for the untreated eyes. It will be seen from these curves that there is no recordable difference between the treated and the untreated eyes. All the untreated eyes showed pathological adaptation curves, however, and in no case did the curves reach the normal threshold level of $10^2 \times 0.6$.

In eight untreated eyes with visual acuity < 0.1 the intraocular pressure was 40–50 mmHg and in the rest untreated and all the treated eyes it was < 40 mmHg.

Rubeosis iridis was present in five treated and in 26 untreated eyes.

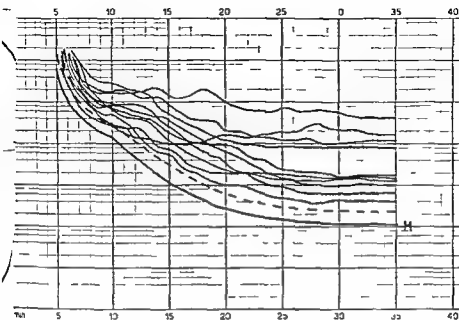


Fig 2

dark adaptation curves recorded in the treated eye at 9 to 11 years after photocoagulation in diabetics between 40 and 60 years old. H = threshold values in healthy individuals in this age range. broken curve represents upper borderline of normal range of threshold values

Discussion

The value of photocoagulation treatment in diabetic retinopathy has been repeatedly documented in extensive well-controlled studies. Up to the present, however, the patients have been followed up for few years only. Experiences from a large American study in 1976 showed that the results for the treated eye improved further with the time that elapsed after the photocoagulation.

Kfemen & Freyler (1979) report their results of a retrospective study comprising 33 diabetics who were followed up for 10 years after treatment by Xenon arc photocoagulation of one eye. The untreated fellow eye showed more severe fundus changes and impaired visual acuity. The authors do not discuss any long term complications of the treatment.

The results obtained in the present study (Table I) show that a general impairment of visual acuity occurred both in the treated and in the untreated eyes.

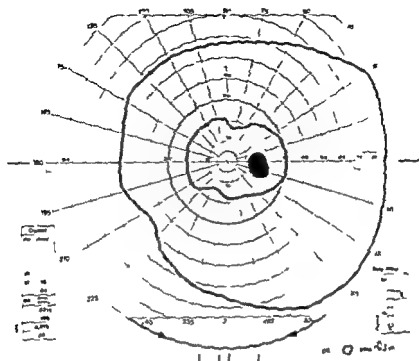


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In eight untreated eyes with visual acuity < 0.1 the intraocular pressure was 40–50 mmHg and in the rest untreated and all the treated eyes was < 30 mmHg.

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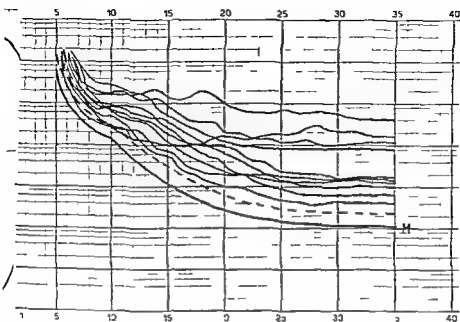


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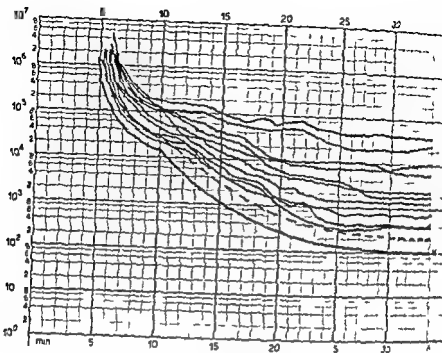


Fig 3

Dark adaptations curves recorded in the untreated fellow eye in the same group as

but that severe visual loss was present in only three treated eyes (4%) after 4 years. Severe visual loss occurred in 18 (25%) of the untreated eyes.

The retinopathy was more marked in the untreated eyes. Proliferative retinopathy, which at the start of the treatment period occurred in 59 of the 73 eyes, was present in 43 eyes at the follow up examination, which means a lower risk of haemorrhage for 15 eyes. In 1979 proliferative retinopathy occurred in 11 of the untreated eyes, i.e. an increased risk of haemorrhage for 11 eyes.

Distinct retinal atrophy with very narrow vessels and some atrophy of the retina were noted in well over one third, or 28, of the treated eyes and in 18 of the untreated. This fact supports the hypothesis that after photocoagulation of large parts of hypoxaemic retinal tissue are destroyed the risk of neovascularisation would diminish and that hence the remaining retinal tissue might improve. One possible cause of the persistence of photocoagulation could be that in some cases it leads to retinal atrophy of the same type as that occasionally seen after diabetic retinopathy of early onset, in which it seems to undergo spontaneous self healing where the metabolic activity is reduced and oxygen demand is lessened. In these cases the

possible that because of reduced numbers of viable cells in the retina there is an insufficient amount of vasoproliferative factor produced to stimulate neovascularization.

A careful analysis of the visual field in 40 of the treated eyes showed minor subjectively unnoticed scotomas in 18. The Diabetic Retinopathy Research Group report (1976) that one year after the treatment 44% of the Xenon arc treated eyes had visual field scores in the range of 240–500 degrees. Frank (1975) reports visual field defects after extensive argon laser coagulation of 24 eyes in which 10 defects not subjectively noted were present in 21 and severe visual field loss subjectively noted in three eyes. In the present material the occurrence of severe visual field defect as a complication seemed to be insignificant. The varying degree of visual field defect between different materials is probably due to more or less aggressive treatment with respect to extent and effect.

The ten patients whose dark adaptation was studied were selected by visual acuity and age for comparison with eyes of healthy controls in the same age range. There was no recordable difference in the appearance of the curves between the treated and the untreated eyes. All the eyes showed pathological curves which were poorer than normal particularly towards the end of the time required for normal dark adaptation. This is in agreement with the observations by Zetterstrom & Litterberg (1973) who examined 20 diabetics with retinopathy before and several months after photocoagulation and found no significant improvement or impairment of dark adaptation. Nor did the adaptation pattern in these eyes seem to have changed after a long observation period (9 to 11 years) on comparison with the untreated eyes. None of the 20 examined patients reported any increased difficulty seeing at night or in getting used to the dark. This observation was confirmed by Jensen & North (1979) who studied the dark adaptation of 35 diabetics.

In conclusion the results in the present followed up material show that even after a long observation period the treated eyes had retained better visual acuity than there were fewer eyes with severe visual loss and that the risk of catastrophic hemorrhage was reduced in comparison with the conditions of the untreated eyes. It did not seem that the treatment seemed to have been followed by any contra-indicating long term complications.

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A PRESSURE LOWERING EFFECT OF RETINAL XENON PHOTOCOAGULATION IN NORMOTENSIVE DIABETIC EYES

BY

SØREN NICOLAI SCHIODTE ERIK SCHERFIG and OLE I NISSEN

In 10 diabetic patients with normal intraocular pressures the effect of monocular panretinal xenon photocoagulation was recorded with the applanating suction cup tonograph applied bilaterally. One month after the treatment the photocoagulated eyes showed an average decrease in pressure of approximately 3 mmHg which was statistically significant ($P < 0.001$) when compared in the untreated fellow eye. On a percentage basis the falls were of a similar magnitude (approximately 20%) as those observed by others after photocoagulation in neovascular glaucoma.

Key words: applanating suction cup tonograph - diabetes - intraocular pressure - photocoagulation

As shown by Laatikainen (1977) that a panretinal xenon photocoagulation has a beneficial effect on the intraocular pressure of eyes with neovascular glaucoma. In the present study we have demonstrated that this effect is not only due to the special effect of the blood circulation of the eye in this severe disease - actually a xenon photocoagulation treatment also reduces the pressures of normotensive diabetic

Material and Methods

In this study one eye was treated while the other eye served as a control. Prior to pre-treatment examination 10 patients were selected (eight males and two females, age 53 years) who had fairly symmetrical conditions regarding intraocular pressure and slight degrees of proliferative diabetic fundus changes. The Goldmann tonograph applied bilaterally was used for the pressure measurement. In order to obtain an accurate comparison between fellow eyes both with respect to pressure levels and amplitudes. All the pressures given are the steady state (equilibrium) pressures obtained by continuous recording with this method (for details see Auer 1972). Presented pressures are the averages of such registrations made before and after interchange (left-right) of the cups. The distribution of the intraocular pressures in the patients appears from Table I columns 3-4.

The photocoagulation of the trial eyes was arranged in two steps with an interval of one month. The same eye was photocoagulated both times. The IOP of both eyes was measured approximately 24 h before and after each photocoagulation. Finally a third IOP measurement was made one month after the second treatment. The non-photocoagulated eye functioned as control eye. A Zeiss xenon photocoagulator model NBO 111 was used. Clinically satisfactory burns were normally obtained by adjusting the intensity of the diaphragm 4-5 units diaphragm I and power output Grundlast III. The patient was positioned supine and special care was taken not to let the burns overlap. The intensity of the light was kept so low that the appearance of double haloes was avoided. Only when the intensity was high enough to produce an immediate whitish bleb immediately followed later by an easily detectable pigmented scar.

All the treatments were performed by one of us and to avoid an asymmetrical effect from the mydriatics (phenylephrine 10% and Mydracil® 0.5%) both eyes of the patient to be treated were anaesthetized with 3 ml 2% lidocaine containing 0.1 mg atropine and 1000 IU Penetrase® injected retrobulbally.

Results

The pressure (IOP) of the experimental eye and the control eye of the 10 patients before and after the treatments are given in Table I. On an average the IOP of the experimental eye was 0.8 mmHg above that of the control eye before the first treatment. However, one month after the first treatment the difference was reversed: the IOP of the experimental eye was now below that of the control eye and this remained so. The IOP of the control eye seemed unaffected.

A statistical treatment (Student's *t* test) of the differences between the IOP of the experimental eyes and the control eyes shows that there was a significant increase in IOP of the experimental eyes one month after the first treatment and one month after the second treatment when compared to the pre-treatment values. From a statistical point of view there was no additional pressure effect from the photocoagulations of the second treatment ($P < 0.05$).

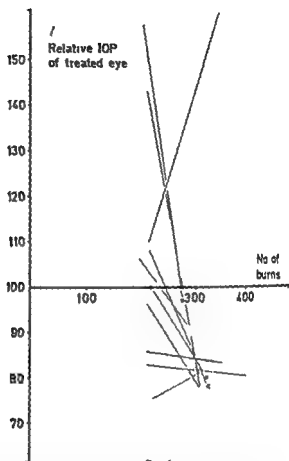


Fig. 1

The acute effect of monocular venous photocoagulation on relative intraocular pressure of the treated eye. Abscissa: number of coagulations. Ordinate: relative pressure of the treated eye expressed by the percentage given in Results (eq. 1). • IOP measured 1 h after the first treatment. × IOP measured 24 h after the second treatment in the same eye. — between treatments: one month.

In order to illustrate this effect of photocoagulations on IOP as expressed by the pressure ratio $IOP_{exp}/IOP_{control}$ after the treatments in per cent of the ratio observed before the first treatment (Figs. 1 and 2)

$$\frac{[IOP_{exp}/IOP_{control}] \text{ after treatment}}{[IOP_{exp}/IOP_{control}] \text{ before first treatment}} \times 100\%$$

— a percentage of 100 means no effect; a percentage below 100 means a lowering effect of the treatment.

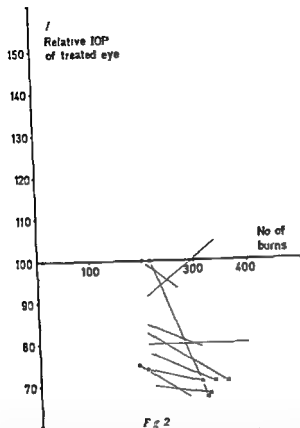


Fig 2

Effect of monocular xenon photocoagulation on the relative intraocular pressure of the treated eye. Abscissa: number of coagulations. Ordinate: relative pressure of the treated eye expressed by the percentage given in Results (eq 1). ● IOP measured one month after the first treatment. x IOP measured one month after the second treatment in the same eye. Time between treatments: one month.

From Fig 1 it may be seen that the acute effect of the treatment varied considerably. Twenty-four hours after placing on an average 200 coagulations per eye, patients showed a relative pressure rise of 7 to 59% in the treated eyes (value of 107 to 159%). Four patients showed a fall of 3 to 25%. In one patient the relative pressure was unchanged. Twenty-four hours after the second treatment (on an average another 122 burns) one patient showed a pressure rise of 62%, another was unchanged, and in the rest (8 eyes) the relative pressures of the treated eyes remained low, though on a slightly higher level than immediately before the second photocoagulation.

The *chronic* changes in pressures were more consistent (Fig. 2). One month after the first treatment eight of the 10 eyes showed falls in pressures of 8 to 30%. Two eyes did not change. When examined one month after the second treatment a majority (9) of the experimental eyes again showed relative falls in pressures to the situation before the first treatment. The decreases were 7 to 39% in the experimental eyes, pressure increased by 6% in the control eyes.

Discussion

One month after treatment with the xenon lamp the photocoagulation probably healed and changed to scars. At this time nearly all of the treated eyes reacted with a pressure fall (Fig. 2). From a pre-treatment level of 13.4 mmHg two photocoagulation treatments caused an average pressure decline of 3.3 mmHg (the control eyes did not on an average show any change in pressure, see columns 3-4 with 11-12 in Table 1).

To explain a pressure decrease of 3.3 mmHg a very considerable decrease must take place in the aqueous formation or in the resistance of the aqueous outflow channels, because a depression of these parameters even to zero would make the IOP of the recumbent patient to fall only to a value near the episcleral pressure, viz. about 10 mmHg. Accordingly, a panretinal photocoagulation must cause a profound disturbance in the inflow-outflow mechanisms of the aqueous humour.

On a percentage basis the pressure decreases in our experiments are of the same magnitude as those seen after photocoagulations in neovascular glaucoma (Käyser 1977).

A number of possibilities which are under investigation may explain the pressure fall. The immediate necrosis or the later scarification due to the burns might lead to a larger choroidal arteries or nerves supplying the ciliary body resulting in a depression of the aqueous formation. Other findings not published here indicate extensive damage to choroidal and ciliary structures, a reduction of the ocular pulse amplitude, a fall of the corneal sensibility, a dilated pupil in the dark, light and a reduced range of accommodation.

Rogell (1979) observed a high frequency of internal ophthalmoplegia after panretinal photocoagulation with the argon laser. Furthermore, histological examination of choroidal casts from fresh xenon photocoagulated rabbit eyes revealed a destruction of the choriocapillaries, larger choroidal vessels in addition to a destruction of the choriocapillaries (Rogell 1979).

An increased hydraulic conductance of existing or new formed pathways

ous humour from the eye was a second explanation for the pressure fall after photocoagulation. Such increase might be due to an absorption of aqueous across the choroidal retinal cicatrices or to a traction on the trabecular work from a scarified choroid. A third explanation is a fall in the pressure of leral and aqueous veins caused by a lowered blood perfusion of the eye. It tonographic studies may throw light on these problems. It is possible that also the retrobulbar anaesthesia might play a role in the pressure. One possibility is that the retrobulbar anaesthesia by itself is responsible. This is an unlikely explanation since it has been shown that the pressure seen in both glaucomatous and normal eyes after administration of bulbar anaesthesia is only of very short duration, not more than one hour and 1/2 (Snydacker et al. 1954, de Roeth & Carroll 1955, Mees 1940). Another possibility is that the retrobulbar anaesthesia intensifies the effect of xenon coagulation. Hørvén (1978) and Pohjanpelto (1979) described a pronounced ease in the ocular pulse amplitudes immediately after a retrobulbar injection of cal anaesthetic. Supposing that this indicated a lowered choroidal blood flow created by the intraorbital presence of anaesthetic fluid, an eye treated in a bulbar anaesthesia may be more extensively affected by a photocoagulation treatment because an insufficiently perfused tissue will be less resistant to heat. Investigations on the effects of treatments using the argon laser with and without bulbar anaesthesia might give an answer. The immediate effect of the coagulations is known to be hyperaemia and dilation of the choroid (Diddie & Ernest 1977, Kolker & Hetherington 1976). This may explain why we find relative increases of IOP 24 h after the treatment in a number of patients (Fig. 1). These acute effects of photocoagulations will not be further discussed in this article.

Acknowledgments

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ATERAL RECORDING OF HUMAN INTRAOCULAR PRESSURE WITH AN IMPROVED APPLANATING SUCTION CUP TONOGRAPH

BY

OLE I. NISSEN

An improved applanating suction cup for continuous recording of human intraocular pressure is described. The new cup adheres better, is easier to calibrate, and is simpler to handle, mainly due to a different curvature of the applanating surface. The interspace between the applanating and the applanated surface, in which the pressure is measured, is continuously flushed with saline from a flow system (Intraflo®) connected to the dome of the pressure transducer. The suction that attaches the cup to the cornea — as in the previous model — provided by a saline filled tube hanging from the cup. In 51 human cadaver eyes the calibration for static pressures and pressures oscillating up to 194 cycles per min was intraocular pressure = 0.9 × cup pressure - 3 (mmHg), $r = 0.995$. The cup reduced ocular rigidity by 5% only and increased the intraocular pressure transiently by 4 mmHg when applied. Simultaneous bilateral recordings were obtained in 400 normo- or hypertensive eyes from 206 subjects. The cup was atraumatic and the method can be strongly recommended when accurate bilateral pressure recordings are desired for periods up to one h in supine subjects. At intraocular pressures above 35 mmHg the tonograph may underestimate the pressure under certain unfavourable conditions.

Key words: continuous pressure measurements — intraocular pressure — instrument — pulse amplitudes — tonograph

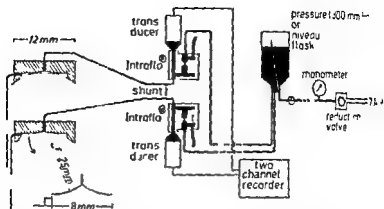


Fig 1

The setup for bilateral pressure measurement in human subjects. The two suction cups shown to the left, the position of the cornea is indicated underneath the upper cup. The applanating surface is shown in detail below: the curvature is exaggerated for clarity; the radius of the correct curvature. The resistance capillary of the Intraflo unit is bypassed by pulling its rubber rod: the bypasses are indicated by dashed lines. Seven atmospheric air pressure was available in the pressure system of the hospital. The shunt is used for calibrations.

The applanating suction cup tonograph (Nissen 1977a) is suitable for bilateral recording of the intraocular pressure in supine subjects for periods of up to 30 minutes. The two lucite cups adhere to the corneas by the negative pressures produced by the water columns in tubes hanging from the cups. The improved model has now been tested in 80 eyes of human cadavers and in 206 subjects with normal or elevated intraocular pressures and appears to be an accurate and well tolerated instrument. We will begin by summing up the improvements.

1) While the depth of the earlier cup had to be adjusted in the cornea by turning its central applanation part in a screw thread, the new, simpler cup is made in one piece. A standard cup fits the eyes of five sixths of the patients; a flat or a deep cup has to be used for the remainder.

2) Compared to the previous model, the new cup bends the cornea less at the periphery of the applanated surface due to the steeper curvature of the applanating surface (Fig. 1). This means that the repulsive force caused by the rigidity of the cornea substance becomes less, i.e. at the same intraocular and suction pressures the cup adheres better.

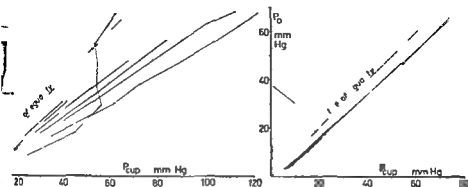


Fig 2

effect of change in curvature of the applanating surface. Left: A plane applanating surface was mounted in the screw cup described by Nissen (1977a) and screwed in stepwise to applanate the surface (bottom curves correspond to small applanated surfaces). Right: The new curved applanating surface is mounted in the screw cup and the procedure repeated. Each set of lines is made in one human cadaver eye. For details see Nissen (1977a) and the introduction.

The curvature of the applanating surface is also designed to make the calibration line independent of the diameter of the applanated area: this means that the same calibration is used with the three cups mentioned above. The effect of making the surface cone shaped and convex (Fig. 1) may be explained thus. We know that if the applanating surface is plane and the applanated area small, a relatively large part of the pressure in the interspace is used to bend the cornea; a smaller part to neutralize the intraocular pressure; vice versa if the applanated area is large (Fig. 2). Therefore, if the applanating surface is designed to bend the cornea less at all applanated areas than at large areas, this effect is counteracted and the calibration lines for different applanated areas may be brought to fall on top of each other (Fig. 2 right).

The present model of the cup has only one central connection. This makes the handling of the cup easier. The infusion pump is replaced by a compact continuous flow system (Intraflo®) which fits the transducer dome and is available in sterilized sets.

Methods

The general principle of the tonograph has been described in the account by Aasen to which the reader is referred.

The dimensions of the new cup are shown in Fig. 1. In the deep and the flat corneal surface was displaced respectively up and down by 0.175 mm. The two left cups were each connected to a pressure transducer (Saham P33 Db) and a piece of Silastic tube (id 0.61 mm, od 1.13 mm) a T fitting and a stopcock (Intraflo® Sorenson Research Co.) fitting the Luer connection of the transducer. When the two flush systems were fed with a sterile Ringer's solution (per liter: sodium chloride 156 meq, potassium 4 meq, potassium 46 meq, calcium 2 meq, magnesium 1 meq, approx. match 50 µl/min) were delivered to each cup for measurement of the difference between appplanating and appplanated surfaces. With this flow of fluid the pressure from the transducer to the cup was 0 to 0.4 mmHg. The two T fittings were connected to a short piece of Silastic tube (the shunt in Fig. 1) supplied with a clamp. This shunt was removed during the measurements both transducers were exposed to the same pressure. The two recorder pens could be adjusted exactly to the same level in order to agree on accuracy of a subsequent comparison between the two eyes.

During the electric adjustment of the transducer recorder systems before the experiments the common pressure flask was used as a level reservoir. Then the anterior chamber was opened to the atmosphere through the stopcock, the tubes to the cups were pinched off, the shunt between the T fittings was opened, and the resistance capillary of one of the flush units was bypassed.

Pieces of Silastic tubes 95 cm long and of the same dimensions as those mentioned above emerged from the sides of the cups and hung down by 90 cm. They created a negative pressure of approximately 15 mmHg in the lateral spaces which kept the cups on the cornea (Fig. 1, Aasen 1977a).

The transducers were connected to a two channel Kompensograph® servorecorder. The frequency response of the tube transducer servorecorder system allowed a square wave pulse amplitude of the living eye. By means of a pressure generator producing different frequencies and of a real scale magnitude we registered a reduction of only 10% amplitudes at 10 cycles/second.

When static pressure calibration curves were determined on human cadaver eyes, the eye was studied at a time when needles in the anterior chamber of the eye was connected to a level flask and to the same transducer as the cup (described by Aasen 1977a). A zero pressure level was defined as that of the anterior chamber.

The capability of the eye-cup-recorder system to measure oscillating pressures was tested on human cadaver eyes by recording simultaneously the pressure in the cup P_c and in the anterior chamber P_a (two transducers were used) during pressure oscillations produced by pulse pressures in the eye produced manually by an intermittent external compression of the eye.

The volume compliance of the eye sphere without a cup applied was tested by injections (and aspirations) of 5 µl of saline into the anterior chamber of the cadaver through one cannula while recording P_0 by another (with the connection to the needle closed).

The pressure recordings on volunteers and patients were made as previously described (Aasen 1977a). The local anaesthetic (0.4% oxybuprocaine) without preservative was used before and every 15 to 20 min during measurements. During the setting of the recording

the shunt connecting the transducers was clamped and the usual 20 μ l/min of balanced solution flowed to each cup both lying in the internal canthus of the eyes. Two types of tractors gave minimal compression of the globe: the Barraquer model (e.g. Storzments No. E-1106-S) equipped with a loop of silk thread preventing the branches from bending too much and putting strain on the lids, and the small Cook model (e.g. Klemments No. 29901). The increase in intraocular pressure produced by these speculae was noted during the recordings by lifting and gathering the branches a little and free them touching the globe and pulling the eye lids.

Results

The calibration line of the standard cup for static pressures is shown in Fig. 3. The regression equation was computed for P_0 values below 50 mmHg by the method of least squares. The equations of the calibration lines for the deep and the flat cup were obtained similarly and are given in the legend. The three lines are hardly distinguishable in a drawing. Forty eyes from 22 human cadavers were used in this part of the study.

The cup was able to measure oscillating pressures correctly. In 32 series of simulated pulse pressures on 11 human cadaver eyes (one single and five pairs) the difference between the artificially produced pressure amplitudes in the anterior chamber and the corresponding pressure amplitudes in the central saline disc of the cup was $\Delta P_{\text{cup}} = 0.896 \pm 0.043$ (s.d.) $n = 32$, a ratio corresponding closely to the slope of the calibration line (Fig. 3). The oscillations were produced at frequencies ranging from 48 to 124 per min in the different series. The ratio $\Delta P_0/\Delta P_{\text{cup}}$ was affected by these frequency variations.

The immediate effect on the intraocular pressure (P_0) of attaching the standard cup to the cornea with the usual suction pressure of 20 cm of water column was measured in 9 pairs of eyes and one single eye of human cadavers disconnected from the level reservoir. Like other cornea touching tonometers the suction cup deformed the eye, therefore P_0 increased. In the P_0 intervals 5–10, 11–20 and 30 mmHg an average increase of 3.9 mmHg (range 2–6 mmHg) was observed. The number of observations within each pressure range was $n = 19$. The average increase in the interval 31–40 mmHg was 3.5 mmHg (range 1.5–7 mmHg). The 17 fellow eyes responded with approximately the same increase, the mean difference in the response being only 1.0 mmHg (range 0.1–2.0 mmHg). (The weight of the cup 0.4 g was too small to increase P_0 . This was tested by putting the cup on the cornea without applying suction: no measurable intraocular pressure increment ensued.)

How the cup affected the volume compliance of the eye sphere was estimated

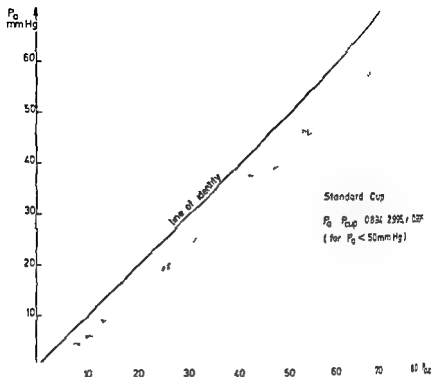


Fig. 3

The calibration line for the standard cup calculated by the method of least squares from 460 measurements on 39 human cadaver eyes. Abscissa: The pressure in the space between appraising and applanated surface (P_{cup}). Ordinate: The pressure in the anterior chamber of the eye (P_O). Similar calibration lines were determined for the flat and the flat cup. Their equations were respectively $P_O = 0.869 \times P_{cup} - 7.3$ (mmHg) coefficient correlation $r = 0.996$ $n = 319$ and $P_O = 0.901 \times P_{cup} - 3.03$ (mmHg) $r = 0.991$ $n = 319$.

from 410 single intracameral 5 μ l injections (and aspirations) in the pressure range 10–20, 21–30 and 31–40 mmHg on five pairs of human cadaver eyes. Without cup on the cornea P_O rose on an average by 4.1, 5.3 and 6.3 mmHg respectively. With a cup attached to the cornea the pressure rose 8.6%, 4.8% and 3.5% respectively, indicating a minor increase in the volume compliance. In terms of percentage change in ocular rigidity (as defined by e.g. Duke Elder 1968) the effect of the deformation were falls of 6%, 4% and 4% respectively. The changes were statistically significant ($P < 0.01$).

The effect on the calibration equation of changing the suction pressure or the flow of saline to the cup was just as small as with the previous model and as before.

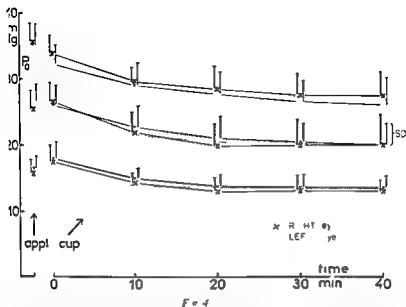


Fig 4 The course of intraocular pressure registrations in 93 supine subjects. The first readings are by Perkins tonometer (left appl) and the curves by the suction cup tonograph (right cup). The dotted curves are hypothetical pressure declines (see discussion).

There was no effect of increasing the temperature from room temperature to 37°C (1977a).

Stable bilateral pressure recordings were obtained from the eyes of 200 outliving human subjects (37 with glaucoma, 49 with intraocular hypertension, 114 volunteers, the others were normotensive patients). 78 were of the female sex. In many of the examinations did not require a recording of a long duration. In 93 subjects the recordings lasted 40 min or more. They were divided into three groups according to the Perkins applanation pressure measured prior to the recordings: 10–20 (n = 23), 21–30 (n = 43) and 31–40 mmHg (n = 27). Within each group the corresponding suction cup recordings were averaged at times 0, 10, 20, 30 and 40 min. The result is shown in Fig 4. The ordinate is the Perkins tonometer P_0 pressure, or the P_0 recorded by the suction cup tonograph using the following common calibration for all three cup tonographs (compare Fig 3): $P_0 = 0.9 \times P_{cup} - 3$ (in mmHg). In Fig 5 are shown 348 comparisons between the Perkins applanation readings and the start P_0 .

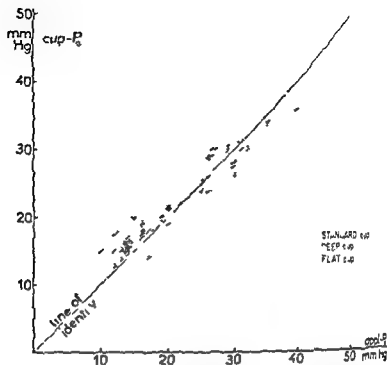


Fig 5

The correlation between the Perkins tonometer readings (abscissa) and the suction cup measured with the cup (ordinate) using the usual calibration $P_0 = 0.9 \times P_{cup} + 1$ mm. The former measurements preceded the latter by 9–10 min.

pressures measured by the suction cup (using the calibration above) for the recordings had to be made with the deep cup and with the flat cup the pressure were done with the standard cup.

The pressure amplitudes due to the beats of the heart measured with the suction cup averaged 2.04 mmHg (range 1.5–3.2 mmHg) in 17 normotensive medical students.

During recording the cup could slide to an eccentric position on the cornea; the two cups could change places without affecting pressure levels or pressure amplitudes. Attempts to extend the period of measurement beyond 50–60 min were unsuccessful due to inconvenience referred to the lid retractors and the lack of freedom of eye movements. The cup never produced more severe corneal abrasions than those seen after a few Goldmann applanation readings, and discomfort afterwards when the effect of the local anaesthetic had disappeared was normally limited to a couple of hours sensation of a conjunctival foreign body.

contact of the lid specular with the globe resulted in a mean increase in P_O of 1 mmHg (range 0–2 mmHg $n = 17$) due to compression – less in old persons and at high intraocular pressures

Discussion

Experiments on eyes from human cadavers show that the new tonograph properly calibrated (Fig. 3) records static intraocular pressures as well as dynamic pressures at least up to a frequency of 124 cycles/min. Since there is no reason to believe that the calibration is valid in living eyes we are convinced that we are dealing with an instrument that is able to record correctly both pressure and pulse amplitudes in the eyes of the patients. The use of the pressurizing shunt during an actual measurement permits an adjustment of the tonometer pens to exactly identical positions at identical pressures. This new instrument is extraordinarily sensitive to small pressure differences between eyes as shown in studies with an experimental eye and a control eye (Schneiders 1970).

The applanating suction cup deforms the eye somewhat more than a Goldmann tonometer. Thus the standard cup produced in living eyes a pressure of 3.9 mmHg (at stop-cock) pressure of cadaver eyes of 3.9 mmHg at intraocular pressure of 31 mmHg and increments of approximately 3.5 mmHg at pressures up to 40 mmHg while Goldmann's or Perkins tonometer measures pressure by only 0.4 mmHg in the lower and 0.8 mmHg in the higher range (calculated from a volume displacement of 0.5 μ l Duke Elder 1956, rigidity of $E = 0.0215$).

Accordingly we would expect that in live normotensive eyes the pressure measured by the standard cup were on average 3.5–4.0 mmHg lower than the Perkins applanation pressure just recorded. In the high pressures the difference should be approximately 4.5–5.0 mmHg. The standard cup augmented the intraocular pressure by about 1 mmHg. Therefore with the deep cup the figures should be 1.5–2.0 mmHg. In the high pressure range and for the deep cup the difference should be 2.0–2.5 mmHg. On all occasions (Fig. 5) a few of the points were considerably below the line of identity. This may have been due to the higher pressures more than one trial was frequently required and sometimes the standard cup had to be replaced (see Fig. 5) because the comparatively large app-

the former resulted in a repulsion by the tense cornea. This added to manipulations and delayed the start of a successful recording probably more prominent the so-called pressure lowering effect of repeated applanations (Moses 1975). In a few cases the applanated surface might have been irregular. For instance, this might happen when an astigmatism in an eye with an eye hypertension necessitated a shift to the deep cup with the result that the applanated area became small and irregular. In normotensive eyes a rather pronounced astigmatism was compatible with measurement with the standard cup.

As the (closed stop-clock) pressure in the eyes of cadavers rose 3 to 11 mmHg when the cup was put on, theoretically we should expect that in live eyes of such magnitude was gradually lost as the pressure decays towards the venous level. But the fall was actually 5–6 mmHg (Fig. 4). The pressure on the lid from the lid speculae can explain approximately one third (0.6 mmHg) of the discrepancy between the predicted and the actual pressure decrease. The remainder being due to a transient increase in the tonus of the eye lids when the speculae are placed and/or to the fact that the subjects relaxed when they were examined. Examination was painless. Not rarely the examinee tended to fall asleep and showed itself immediately as a decrease in the pressures of both eyes.

In live normotensive eyes the pressure reached its equilibrium level in 15 min. In hypertensive eyes in less than 20 min or 30 min (Fig. 4). Such a pressure fall harmonizes with our ideas of the magnitude of the flow of aqueous humour and the volume compliance of the coats of the eye (making allowance for other minor factors also taking part in the pressure fall as mentioned above). To illustrate this in Fig. 4 dashed curves are drawn which are calculated from the integrated Friedenwald equation (Davanger & Holter 1967; Nissen 1971) on the basis of outflow conductances of 0.06, 0.12 and 0.25 $\mu\text{l min}^{-1} \text{mmHg}^{-1}$ (from the top) and an ocular rigidity of $E = 0.0915$. In the calculations we suppose that the sudden volume displacement produced by the appplanation of the cup is equivalent to a step input, e.g. an injection of a volume of fluid into the eye, and also that the ocular rigidity is unaffected by the presence of the cup, which is almost true (see Results).

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ELECTRORETINOGRAPHY BY SKIN ELECTRODES AND SIGNAL AVERAGING METHOD

BY

EILA MUSTONEN and ILMAR SULG

In the investigation of suspected visual disorders ERG is of considerable value. A simplified method for clinical electroretinography by non-corneal electrodes is presented. The latency and amplitude values were measured in 100 normal subjects. Using skin electrodes and signal averaging we can record without discomfort to the patient an electroretinogram with a waveform and time relations similar to those obtained by corneal electrodes. Non-corneal ERG is useful for infants, children as well as sensitive adult patients and is also applicable after recent eye surgery and in cases of infection or injury of the anterior segment. Since non-corneal electrodes do not obstruct the stimulus light or cause refractive change they are advantageous when ERG is recorded simultaneously with visually evoked cortical response (VER). When it is important to stimulate only one eye at a time in flash VER the adequacy of the cover is verified by the absence of an ERC from the covered eye.

Key words: digital computer—ERG—signal averaging—skin electrodes VER

Motokawa & Mita (1942) reported that the ERG could be recorded from non-corneal electrodes, one attached to the bridge of the nose and the other temple near the eye being investigated, but such small signals are usually masked by larger amplitude background activity. The first averaging of ERG was carried out in 1961 by Armington et al. with an analog averager using condensation. Tepas & Armington (1962) reported that averaged potentials picked

des near the canthi of the eye have a waveform similar to the ERG recorded in the eye with a contact lens electrode. Jacobson et al (1962) gave the first report of the use of digital computer technique for recording clinical corneal electroretinogram. Nagata & Jacobson (1966) found that the amplitude of the averaged ERG recorded from the skin electrode was greatest when the active electrode was placed on the lower lid and the reference electrode on the earlobe. The waveform and the time relations were the same as those recorded with a contact lens electrode. Jacobson et al (1966) compared non-corneal skin electrodes with a contact lens electrode and a hook electrode. The eyelid retractor hung over the lower lid. They used a pair of silver disc electrodes attached about 5 mm from the external and internal canthi of the eye. The temporal electrode used as the active one. The amplitude of the response recorded with the hook electrode was approximately three times as high as that of the contact lens electrode and about 60% of the magnitude obtained from the contact lens electrode. The blink artifact was more manifest in the record from the hook electrode than from the skin electrode or from the contact lens electrode. Recent advance of averaging computers has made it possible to clinically evaluate visually evoked responses (VER) obtained from occipital electrodes with flash pattern reversal stimulation. We needed a comfortable and easy method for recording simultaneously retinal responses (ERG). Non-corneal electrodes are also applicable in children without general anaesthesia or sedation and averaging technique with digital computer has made it practicable to record ERGs from skin electrodes. Because one indication for simultaneous ERG and VER studies was the preoperative examination of the patients referred for vitrectomy, we especially studied normal values of retinal responses to bright light flashes. The normal range of ERG parameters recorded with our non-corneal technique is reported in the present paper.

Material and Methods

Twenty-two normal subjects (43 eyes), 19 women and 3 men between 21 and 58 years of age were examined for measuring normal amplitude and latency values of ERG by non-corneal skin electrodes and signal averaging technique. Their pre-examination included visual acuity tests and refraction. Friedmann and Goldmann visual fields, Farnsworth Munsell 100-hue test as well as slit lamp and fundus examination. After pre-adaptation in weak room illumination ($\approx 5-3$ lux) for 25 min, followed by 5 min in darkness, the subject was stimulated with 10 flashes (100 lux) of 10 mseconds duration and intensity of 0.5 joule from an Elema Siemens photostimulator (EMT 720) with a lighted area 160×30 mm, giving white light flashes with 15-second intervals 90 cm in front of the

Table 1

The latencies (measured from the stimulus-on) and the amplitudes of a- and b-wave (b-wave amplitude measured from the peak of the a wave) in non-corneal ERG from lower lid skin electrodes in 22 normal subjects (43 eyes) with stimulation by 15 second intervals and same parameters in 15 of normal subjects (29 eyes) with stimulation by 32 flashes at 2 Hz frequency. Flash intensity 0.2 joule

	Latency (msecond)						Amplitude (µV)					
	a wave			b-wave			a wave			b-wave		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
<i>10 flashes at 15 second intervals (0.2 joule)</i>												
Right eye	10.0 ± 2.19		(22)	39.8 ± 2.76		(22)	11.6 ± 6.50		(22)	31.5	13.2	
Left eye	9.5 ± 1.42		(21)	39.6 ± 3.21		(21)	10.3 ± 5.42		(21)	33	13.4	
Average												
R & L eye	9.8 ± 1.81		(43)	39.7 ± 2.99		(43)	11.9 ± 5.96		(43)	32.6	13.3	
<i>32 flashes at 2 Hz frequency (0.2 joule)</i>												
Right eye	9.3 ± 1.49		(15)	39.6 ± 2.71		(15)	6.4 ± 3.35		(15)	24.0 ± 11.6		
Left eye	10.2 ± 2.15		(14)	39.8 ± 2.19		(14)	8.0 ± 3.9		(14)	22.8 ± 11.6		
Average												
R & L eye	9.7 ± 1.82		(29)	39.7 ± 2.45		(29)	7.2 ± 3.5		(29)	23.4 ± 13.4		

subject's eyes. Pupils were not dilated. Each eye was stimulated separately, the other carefully covered by an eye patch. The adequacy of the cover was verified by the absence of ERG from the covered eye. Another averaged ERG was then recorded in 15 of the subjects (29 eyes) with stimulation by 32 flashes (intensity of 0.2 joule) at 2 Hz frequency. Simultaneous V-ER was recorded. Each eye was again stimulated separately and the other was carefully covered. Further V-ERs were then recorded with reverse pattern stimuli in all normal subjects. Our method and results of V-ER studies will be presented elsewhere.

ERGs were recorded from infraorbital skin electrodes with the ipsilateral earlobe as reference and the usual EEG electrodes at the vertex as the ground ones. Circular disc electrodes 10 mm in diameter were attached by an adhesive ring to the lower lid, the centre of the pupil close to the lid margin and the contact was confirmed by electrode paste.

The primary amplification and recording of ERG was performed on a Elema Siemens EEG-machine (Mingograph Universal) time constant 0.3 sec, frequency cut off from 70 Hz. From the Mingograph the amplified signals were transferred to a 2-channel 1000 points signal analyser/averager (Hewlett Packard 5490). The responses with 200 msec averaging time were documented by means of photographs from the face of the oscilloscope of the signal averager. For more detailed recording an XY recorder may be used.

In a similar procedure one female patient with an enucleated left eye was examined to see whether the electrical field of the healthy right eye extended to the opposite lower lid. So of the normal subjects both corneal and lower lid ERGs were recorded with four different intensities of light (0.0025, 0.01, 0.04 and 0.2 joule respectively) averaging the responses to 10 flashes at 15-second intervals after pre-adaptation to compare the waveform variations. Corneal ERGs were recorded from Lovac contact lens electrode (Medical Top Holland).

Results

Latencies of the a wave and b-wave were manually measured from the on-set to the peaks of the waves. The amplitude of the photopic b-wave was measured from the peak of the a wave. There were more interindividual variations in amplitudes than in latencies. Usually the two eyes of one subject showed quite different amplitudes although interindividual variations were great. The results in normal subjects are given in Table 1 and one example of normal corneal and lower lid ERGs with four different intensities of light is presented in Fig. 1. In the lower lid ERGs the sensitivity was five times that of the corneal curves and respectively background noise was greater in non-corneal ERGs but the waveform and time constants were similar. Our technique with only 10 flashes at 15-second intervals or 2 Hz frequency revealed no measurable ERG from the lower lid electrode of the enucleated left eye when the healthy right eye was stimulated. In Fig. 2 there are curves from the lower lid electrode of the healthy right eye and the enucleated left eye with stimulation by 128 flashes at 2 Hz frequency and the sensitivity was twice that of the usual non-corneal recordings. There are some peaks in the curve from the lower lid of the enucleated left eye but they do not significantly differ from the background noise curves recorded from the two eyes with stimulation by 10 flashes at 15-second intervals while both eyes were carefully covered and the patient did not perceive any light so that it could not be interpreted that the electrical field of the healthy right eye extended to the opposite lower lid. When the right eye was carefully covered no ERG response of course was recorded from the right lower lid electrode while the left enucleated eye was stimulated by 32 flashes at 2 Hz but when the right eye was purposely poorly covered with the patch there was an ERG with a large amplitude and long latency without an a wave showing characteristics of a dark adapted ERG with stimulation by dim light (Fig. 3).

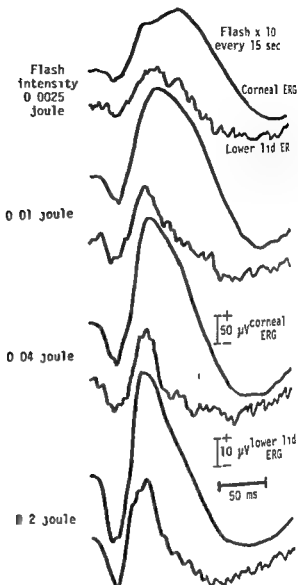


Fig 1

Corneal and non corneal ERGs in one normal subject examined with 10 flashes at intervals using four different flash intensities. Note the difference in calibration.

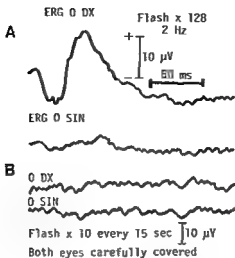


Fig 2

stimulation of the right healthy eye with 128 flashes at 2 Hz frequency. No measurable response from the infraorbital electrode of the left enucleated eye. B The background noise from the two eyes with 10 flashes at 15 second intervals while both eyes were carefully closed and the patient did not perceive any light. Note the difference in calibration signals.

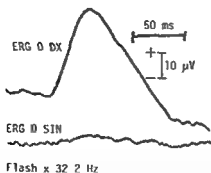


Fig 3

The right healthy eye was purposely poorly patched during the stimulation with 32 flashes at 2 Hz frequency. From the right infraorbital electrode there is an ERG with no a wave and with a long latency and large amplitude b-wave characteristics of a dark adapted ERG with stimulation by dim light. No measurable response from the infraorbital electrode of the left enucleated eye.

Discussion

Tassy et al (1968) examined the electrical field of the eye and found responses from the orbitoinferior skin electrode referred to the temple of the same side. They reported that with averaging technique (150-2100 responses) it is possible to record small responses from the corneal electrode of the opposite eye or from the skin electrodes around the opposite cornea. In one instance in one patient enucleated eye. The amplitude of the response at the cornea of the opposite eye was less than 1/50 of the normal ERG.

Hirose & Jacobson (1968) reported their experience with simultaneous recording of ERG and VER in patients with visual disorders. They used disc electrodes 4 mm in diameter placed on the lower lid at the centre of the lower orbital margin referred to the joined earlobes. Schmidt & Straub (1970) used small EEG-electrodes attached to the inner canthus of each eye and the reference electrode at the forehead. The potentials registered by skin electrodes were 1/6 of the amplitude of the ERG in the conventional way. They could record using 900 flashes/mult from the electrode on the lower lid of an enucleated eye and thus showed the electrical field of the stimulated eye extended to the opposite side.

Harden & Pampiglione (1970), Harden et al (1973) and Harden et al (1974) described the use of a non-corneal nasal bridge electrode when investigating adults and children with a combined procedure of simultaneous ERG, VER and VEP in order to establish the level of suspected visual impairment. They reported that the amplitude of the response recorded from the cornea was 20 times greater than the amplitude of the response from the nasal bridge electrode referred to the electrode on the scalp at the vertex. Stephens et al (1971) reported that the ERG responses from their skin electrodes were 1/3-1/5 of that obtained with the contact lens electrode. Noonan et al (1973) examined the influence of direction of gaze on the human ERG recorded from periorbital electrodes and found that in all directions of gaze except for upward gaze the ERG components were higher in amplitude at the inferior electrode. Nakamura (1975-1978) examined the isopotential lines of the ERG and found that the ERG was widely distributed over the face. The maximum of the scotopic ERG was located on the lower lid where the amplitude was one third or one fourth of that obtained from the contact lens electrode. In his work he used linked earlobes as the common reference.

Marmor (1976) had successfully recorded corneal ERGs in children under 5 years of age without sedation, but Jones & France (1978) preferred non-corneal inferior orbital ridge electrodes for ERG when investigating children and recorded simultaneously VERs. Berry (1976) found 16 repetitive flashes sufficient to obtain good non-corneal ERGs from the lower lid electrodes referred to the electrode at the bony margin of the orbit behind the outer canthus. I did

79) reported their studies on skin-electrode ERG in the closed-eye state active electrodes were placed on the nasal side of the lower orbital margin (Smith's position 3 1971) Giltrow Tyler et al (1978) found that the lower lid electrode produced a larger signal than the nasal electrode even with medial viewing but it was associated with greater interindividual variation and that the electrode at the medial canthus combined with medial viewing gave closest approximation to the corneal ERG

We chose skin electrodes placed on the lower lid at the centre of the lower orbital margin and referred them to the ipsilateral earlobe electrode Signal averaging technique of 10 flashes at 10-second intervals were sufficient to get good photopic responses from non-corneal electrodes This simplified method for clinical ERG without any discomfort to the patient is especially useful for infants children and some adult patients Combining the non-corneal ERG simultaneously with the EEG and EEG we can get information about the level of suspected visual impairment When flash VERs are examined separately from the two eyes the validity of the cover is verified by the absence of an ERG from the covered eye It is possible to reduce the light intensity and lengthen the interval between flashes to get photopic ERGs The same technique the photopic results of which in normal subjects are presented in this report have later been used for examining many different groups of patients and we found it a reliable method in clinical practice for investigating retinal function

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CARBONIC ANHYDRASE ISOENZYME C IN THE HUMAN RETINA

An immunohistochemical study

BY

TIMO KUMPULAINEN

The occurrence of carbonic anhydrase isoenzymes C and B in the retina of the human eye was examined with specific antisera against these enzymes. Immunological analyses were carried out by the double diffusion method of Ouchterlony and the localization of the enzymes was studied by an application of immunoperoxidase (PAP) technique. A large amount of isoenzyme C was detected in tissue sections from the human retina whereas the isoenzyme B was totally absent. Isoenzyme C was considered to be located primarily in the glial elements of the retina correlating with the earlier findings obtained by traditional metal salt methods.

Key words: human retina - carbonic anhydrase - isoenzyme C - immunohistochemistry

Carbonic anhydrase (CA) which catalyses the reversible hydration of carbon dioxide to carbonic acid occurs in mammals in two forms with different activities: so called high activity CA is designated isoenzyme C and the low activity form isoenzyme B. The proportions and functional significances of these CA isoenzymes in various tissues are not fully known (see e.g. Carter 1972). The ratio of isoenzyme C to isoenzyme B in human erythrocytes is about 1:5:7 but it is not possible to define the role of the B enzyme whereas the C enzyme is known to be responsible for vital respiratory functions (Chapman & Maren 1978).

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CA activity has also been reported in various parts of the eye. The histochemical location of the enzyme in the pigment epithelium and glial elements of the retina (Korhonen & Korhonen 1965, Leder 1966, Bhattacharjee 1971, Musser & Musser 1973a, b) suggests a role in carbon dioxide metabolism and ionic changes probably involved in the propagation of the nerve stimuli. The histochemical methods of Hausler (1958) and Hansson (1967) are those most commonly used for the demonstration of CA activity in the retina. These methods are based on the deposition of metal salts at the sites of enzyme activity, but their specificity has been criticized (see Muther 1972, Churg 1973). Since immunological methods seem to offer a highly specific means of demonstrating enzymes and isoenzymes, immunohistochemical methods have been developed for both CA isoenzymes C (HCA C) and B (HCA B) as reported previously (Korhonen 1978, Kumpulainen 1979). The immunoperoxidase method was also used for the demonstration of CA in certain tissues of the rat and the rabbit (Delaunoy et al. 1977, Roussel et al. 1979, Spicer et al. 1979). There is no published immunohistochemical study of this enzyme in the ocular tissues. The aim of the present investigation was to study the occurrence of the CA isoenzymes in the human eye.

Material and Methods

Tissue samples

Three normal human eyes, enucleated originally about 1½ hours post mortem, were obtained from the Department of Ophthalmology, Oulu University Central Hospital. The eyes had been stored in cold physiological saline solution for about three h before being transferred into liquid nitrogen. Tissue pieces from ocular operations were also tested, but the results were discarded. The immunoperoxidase staining procedure was found to be better preserved in an intact bulbus instead of a tissue piece was frozen before cutting with a microtome.

The retinal material for immunodiffusion was obtained by cutting up frozen sections of about 20 µ from the posterior part of the bulbus. In these thick sections the retina, including the pigment cell layer, usually loosened from the choroid and was easy to collect into dry test tubes, where it was allowed to dry. The tissue fluid then being used for immunodiffusion.

Antisera

Rabbit antisera against HCA C and HCA B were used for demonstration of the CA isoenzymes. Rabbit antiserum to ovalbumin served as a control serum for immunohistochemical staining. The preparation of the antisera together with their specificities is reported elsewhere (Kumpulainen 1979).

Immunological analyses

The double diffusion method of Ouchterlony (1968) on agarose plates with barbital buffer pH 8.0 ionic strength 0.1 was used to test the immunoreactivities of the antisera and to demonstrate CA isoenzymes in the retinal material and in the human erythrocyte hemolysate which served as a control sample containing both CA isoenzymes. Human erythrocyte hemolysate was prepared by lysing the erythrocytes with an equal volume of distilled water.

Immunohistological technique

The immunoperoxidase staining procedure for human CA has been described in detail previously (Kumpulainen 1979). Frozen sections of about 11 μ were used for immunohistochemical demonstration of the CA isoenzymes in the retina. These sections were placed on cold glass slides and fixed with -10°C methanol (99%) for 1 min, then removed from the cryostat chamber.

In the immunoperoxidase method the tissue sections were first incubated with bovine serum albumin (Sigma) in PBS (0.85% saline, 0.01 M phosphate buffer, pH 7.4) for 5 min to minimize any unspecific absorption of the antisera. Specific anti-CA I and anti-CA II sera (in control rabbit anti-ovalbumin serum) diluted 1:200 in PBS were then applied to slides for 15–30 min in a moist chamber. The slides were then washed for 30 min in PBS with rotary agitation and incubated for 15–30 min with swine anti-rabbit serum IgG (Dako-immunoglobulins) diluted 1:20 in PBS. After a further washing for 30 min in PBS the slides were incubated with peroxidase anti-peroxidase (PAP) complex (Dako-immunoglobulins) diluted 1:100 in PBS. They were then washed again in PBS for 15 min before treatment with 3,3'-diaminobenzidine (DAB, Fluka, 90 mg DAB/100 ml PBS plus 5 drops of 3% H_2O_2) for 5 min. Finally they were rinsed in distilled water, mounted in glycerol and sealed with cover slips.

The slides were examined with a Leitz Orthoplan microscope and photographed with a Leitz Orthomat camera. They were then rinsed in distilled water to remove the cover slips and glycerol and new photomicrographs were taken after routine counterstaining for demonstration of the different retinal layers.

Results

Rabbit anti-CA serum generated a single, clearly visible precipitation line with the retinal tissue fluid in the double-diffusion analysis, whereas no precipitation line was observed between this and the anti-CA B serum. The intensity of the precipitation reaction between human erythrocyte hemolysate and antisera to both isoenzymes were

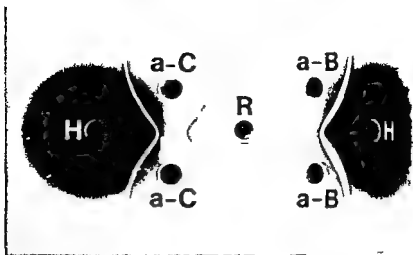


Fig 1

Immunodiffusion Well R contained 12 μ l of human retinal tissue fluid and the cells of human erythrocyte hemolysate. The wells a C were filled with 10 μ l of rabbit anti serum undiluted (upper) or diluted 1:10 (lower) and the wells a B with 10 μ l of anti HCA B serum undiluted (upper) or diluted 1:10 (lower).



Fig 2

Immunodiffusion All centre wells contained 10 μ l of undiluted swine anti rabbit IgG solution. The peripheral wells were filled with 12 μ l of rabbit anti HCA C serum (a-C) on the left, 12 μ l of rabbit anti HCA B serum (a-B) in the centre and 10 μ l of rabbit anti-HCA C serum (a-OV) on the right, diluted 1) 1:10 2) 1:40 3) 1:80 4) 1:160 5) 1:320.

able to the proportions of the isoenzymes in human erythrocytes (Fig. 1). Reactivities between the rabbit antisera and swine anti rabbit IgG were by immunodiffusion prior to their use for the immunoperoxidase procedure. Rabbit anti HCA II and anti-ovalbumin sera showed precipitation lines at twice those required for the rabbit anti HCA C serum (Fig. 2) which indicates that the IgG titres in the diluted anti HCA II and anti-ovalbumin sera were comparable with that of the anti HCA C serum. When the retinal sections were treated with the anti HCA C serum in the first step of the immunoperoxidase procedure a strong peroxidase reaction was seen in the retina (Fig. 3) while sections treated with the anti HCA II (Fig. 4) or anti-ovalbumin sera were negative. The reticular staining of the main part of the retina after the immunoperoxidase procedure with anti HCA C serum corresponds to the reticular arrangement of the fibres. A positive reaction was observed only in the inner segment of the photoreceptor cell layer whereas the outer segment remained unstained. There was only a weak reaction in the ganglion cell layer while the intense colour of the pigment epithelium prevented visualization of the enzymes. The retinal layers in the immunoperoxidase stained sections were identified after staining of the nuclei with cresyl violet (Fig. 5 and 6).

Discussion

Traditional histochemical methods show CA activity in the retina of albino rats and mice to be located in the pigment epithelium and Muller cells (Korhonen & Lahti 1963; Leder 1966) and similar findings have been obtained with other animals (Bhattacharjee 1971; Musser & Rosen 1973a). In the primate retina the cones (but not the rods) exhibited CA activity in addition to the Muller cells by the method of Hansson. In the pigment epithelium the reaction was obscured by pigment granules (Musser & Rosen 1973b). The present results demonstrate the occurrence of the high activity type isoenzyme in the retina of the human eye principally in the glial elements and that part of the photoreceptor cell layer which contains the inner segments of the rods and cones. The role of isoenzyme C may be similar to that probably fulfilled by CA in brain. CA has been localized in the glial cells of the mouse brain while the neuron lacks this enzyme (Korhonen et al. 1964). Later immunohistochemical investigations support this finding (Delaunoy et al. 1977; Roussel et al. 1979; Spicer et al. 1980). It is suggested that in the nervous tissue carbon dioxide formed in the neurons diffuses into the glial cells where it is converted to bicarbonate by CA. Thus CA also influences the sodium and potassium equilibrium of neuronal elements (see Korhonen et al. 1964; Carter 1972) and may thus have an effect on



Fig 3

Figs 3 and 4

Human retina after staining by the immunoperoxidase method using the rabbit anti HCA C serum in Fig 3 and rabbit anti HCA B serum in Fig 4 (x150)

the propagation of nerve stimuli. CA is generally regarded as a cytoplasmic enzyme, being readily soluble after cell disruption (see Lindskog et al 1971). The considerable amount of HCA C which appeared in the soluble portion of the retinal tissue after thawing suggests that there is no substantial binding to the subcellular structures. HCA B was not detected in the retinal tissue fluid using the immunoperoxidase method. This may mean that this enzyme may be more tightly bound to the retinal material, but in such a case it should still give a positive reaction in the immunohistochemical staining. The lack of HCA B in immunodiffusion also indicates that there was no contamination with blood. Methanol and other alcohol fixatives are often employed in immunohistochemistry as they often preserve at least some of the antigenicity of the enzyme proteins (see Sternberger 1974). Although the morphology would be better preserved with aldehyde fixation, methanol is

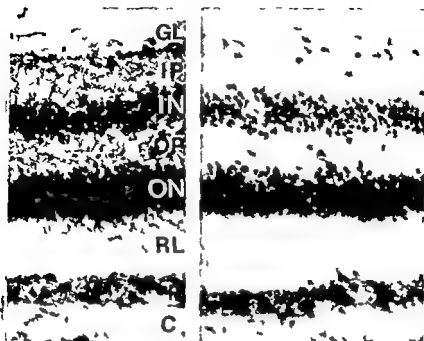


Fig 6

Figs 5 and 6

sections as in Figs 3 and 4 after staining with hematoxyline for demonstration of the layers GL layer of ganglion cells and optic nerve fibres IP inner plexiform layer IN inner plexiform layer ON outer nuclear layer RL receptor cell layer P pigment cell layer C choroid X 180

as a fixative instead of aldehydes because preliminary observation had shown aldehydes destroy the antigenicity of HCA C. Attempts were also made here to demonstrate HCA C in other parts of the eye. Better fixation methods are required however before an accurate immunohistochemical demonstration of CA in the ciliary and in the endothelium of the human cornea for instance is possible. Preservation of the antigenicity of animal CA appears to be better because immunohistochemical studies on animal CA in aldehyde fixed and paraffin embedded material (except ocular tissues) have been reported (Delaunoy et al 1977, Sel et al 1979, Spicer et al 1979) and therefore immunohistochemical demonstration of CA in the eyes of certain albino animals has been subjected to further fixation.

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HISTOCOMPATIBILITY (HLA) ANTIGENS IN CAPSULAR GLAUCOMA AND SIMPLEX GLAUCOMA

BY

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HLA antigen typing of 39 patients with capsular glaucoma and 40 with simplex glaucoma showed a significantly higher frequency of Bw35 in the capsular glaucoma group compared to a control group of 98 blood donors. In the simplex glaucoma group Bw35 was less frequent and the difference was not significant.

Keywords: capsular glaucoma - simplex glaucoma - HLA antigen.

During recent years there has been an extensive search for associations between antigens of the major human histocompatibility antigen system (HLA) and specific diseases and several eye diseases have been shown to be correlated with HLA antigens reviewed by Rahu (1979). Patients with open angle glaucoma reported to have significantly more HLA B7 and B12 and it has been suggested that occurrence of these antigens is an indicator of the development of visual loss (Shin et al 1977). Aviner et al (1976) found that Bw37 was more frequent among patients with open angle glaucoma in a material from the United States against 23% in a control series.

On the contrary Damgaard Jensen & Kussmeyer Nielsen (1978) could not confirm these observations in a Danish material. They found no significant difference from a large control series. Bw35 was present in 10% of the open angle glaucoma patients.

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s against 13% of the control group and B7 and B12 also had frequencies at of the control group Rich et al (1978) found no significant difference in glaucomatous and control populations in incidences of B7 B12 Bw35 A3 B11 Kass et al (1978) found in one white population with primary open angle glaucoma a significant decrease in HLA A1 and a significant increase in antigen B7 and Bw22 These differences could not be confirmed in a white population with primary open angle glaucoma and they suggested associations of A and B loci of the HLA antigen system and primary open angle glaucoma may arise by chance or may be true only in certain ethnic groups David et al (1979) found no differences with regard to the frequencies of HLA antigens and groups of open angle glaucoma and ocular hypertension

The aim of the present study was to compare the occurrence of HLA antigens in glaucoma and simplex glaucoma respectively to a control group of the region

Material and Methods

Forty nine patients with typical open angle glaucoma were selected forty of them with simplex glaucoma in one or both eyes and thirty nine capsular glaucoma The criteria of glaucoma were pathological high intra-ocular pressure (more than 30 mmHg without therapy) together with typical glaucomatous disc cupping and visual field defects with characteristic glaucomatous defects The patients of the investigation were selected before their HLA antigens were known The patients went through compatibility antigen typing by lymphocyte microcytotoxicity technique The control group consisted of 980 blood donors They were tested with the same criteria at the same laboratory Department of Blood Center University of Umeå The differences between the groups were statistically tested with the chi-square test To compensate for the large number of antigens tested (15) the *P* value was corrected by multiplication with 15

Results

The frequencies of 15 HLA antigens tests are presented for the simplex glaucoma group (Table I) and the capsular glaucoma patients (Table II) A significant higher frequency of Bw35 was found in the capsular glaucoma group 28% compared to 10% in the control group ($P < 0.0075$) In the simplex glaucoma group there was no difference for Bw35 which did not deviate significantly from the control group In addition no slight deviations were found (Table I & II)

Table I

The frequencies of different HLA antigens in a group of patients with angina (n=40) and in the control group (n=985)

HLA antigens	No of subjects with antigens	%	Control group	%	P	
A1	8	20%		19%		
A2	25	63%		58%		
A3	15	38%		31%		
A9	5	13%		16%		
A11	4	10%		13%		
Aw26	5	13%		19%		
B5	1	3%		13%	<0.05	
B7	11	28%		36%		
B8	9	23%		18%		
B12	16	40%		98%		
Bw15	10	25%		95%		
Bw22	2	5%		4%		
Bw27	4	10%		16%		
Bw35	7	18%		9%		
Bw40	8	20%		19%		

Table II

The frequencies of different HLA antigens in a group of patients with angina (n=39) and in the control group (n=985)

HLA antigens	No of subjects with antigens	%	Control group	%	P	
A1	5	13%		19%		
A2	26	67%		58%		
A3	6	15%		31%	<0.05	
A9	12	31%		16%	<0.05	
A11	2	5%		13%		
Aw26	4	10%		12%		
B5	6	15%		13%		
B7	8	21%		36%		
B8	5	13%		18%		
B12	11	29%		98%		
Bw15	7	18%		95%		
Bw22	1	3%		4%		
Bw27	6	15%		16%		
Bw35	11	28%		9%	<0.001	
Bw40	10	26%		19%		

Discussion

are several problems and sources of error when studying the frequencies of genes. Racial stratification of the population, statistical errors and selection of patients after HLA typing might be mentioned. We have tried to avoid these by selecting the patients before HLA typing and the patients and the control group live in the same geographical area. The control group, however, is smaller than the glaucoma patients. It is not known if this might affect the results. Specific Bw35 sera was not available, mixed sera of B5 and Bw35 was used. If B5 is found in B locus, Bw35 can exist together with B5 and not be discovered. A false low incidence of Bw35 might have been found, but this possible estimation of Bw35 is probably the same in all three groups and will not affect the conclusion.

In this study we have found a significant deviation of HLA Bw35, which was more strongly represented in the capsular glaucoma group than in the control group. Iwajiri & Jensen (1976) found a higher frequency of HLA Bw35 in capsular glaucoma. Aviner et al. (1976) found a higher frequency (18% against 9% in the control group) but this difference was not significant, perhaps due to a smaller material. However, most patients with capsular glaucoma do not have the Bw35, thus the connection between HLA and the disease is weak.

The great variation between the reports of connection between open angle glaucoma and specific HLA antigens might depend on different criteria for the diagnosis of glaucoma. In this investigation we have endeavoured to include only patients with typical glaucomatous damage; doubtful cases were excluded before HLA typing.

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LYMPHOCYTE SUBPOPULATIONS IN PERIPHERAL BLOOD AND CEREBROSPINAL FLUID FROM PATIENTS WITH ACUTE OPTIC NEURITIS

BY

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Ten patients with acute optic neuritis (AON) were examined for T and B lymphocytes in blood and cerebrospinal fluid by means of rosette techniques. The percentage of T lymphocytes in blood was significantly decreased ($34 \pm 3\%$) compared to controls ($66 \pm 2\%$). Absolute numbers of T lymphocytes and relative and absolute B lymphocyte concentrations were not significantly different from controls. The cerebrospinal fluid (CSF) T lymphocyte percentage was significantly increased (88.8% compared to 78.0% in controls). The relative CSF immunoglobulin G concentration was elevated in four patients (40%). On agarose gel electrophoresis bands in the gamma globulin region were found in two patients (20%).

Key word: optic neuritis – cerebrospinal fluid – peripheral blood lymphocyte subpopulations

Optic neuritis (AON) is the initial symptom in 10–20% of patients with multiple sclerosis (MS) (Leibowitz et al 1966). In adults most cases of AON are manifestations of MS (Taub & Rucker 1954; Hutchinson 1976). Immunological mechanisms have been suggested to be a factor in the etiology of both disorders (Mata et al 1977; Perkin 1979).

Recent reports suggest disturbances in the distribution of B and T lymphocytes in blood and cerebrospinal fluid (CSF) from patients with AON (Kam Hansen et al 1978; Traugott 1978). We have earlier demonstrated changes in lymphocyte populations in blood and CSF from patients with active MS (Næss & Nyland 1979). The present paper reports the results of investigations on lymphocyte populations in blood and CSF from patients with AON compared to patients with active MS and controls.

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Materials

Patients

Group 1 consisted of 10 patients with AON admitted to the Department of Ophthalmology. 2 males and 8 females, aged between 18 and 51 (mean 34 years). The diagnosis was based on medical history and clinical investigations (history, visual acuity, visual fields and color vision). The neuritis was retrobulbar and intraocular (papillitis) in 3 cases. Two patients had an earlier episode of neuritis. The affection was bilateral in 3 and unilateral in 7 patients. Clinical neurological and neuroradiological examinations were performed in all patients. Patients with additional clinical features suggesting MS were excluded from the group.

Group 2 consisted of 21 patients with active MS (Næss & Niland 1982) admitted to the Department of Neurology. 8 males and 13 females, aged between 20 and 60 (mean 36) years. Fifteen of the patients had a history of visual disturbances.

Controls

For peripheral blood studies, 30 healthy persons were selected from the hospital staff. For CSF examinations, 17 patients with headache and psychoneurotic disorders admitted to the Department of Neurology were used as controls. No organic cause for the complaints were found and the cell counts, protein and IgG concentrations were normal.

Blood samples from 7 AON patients were obtained within 2 weeks (mean 10 days) of the acute visual blur. Blood from the other 3 patients were examined after 1, 14 and 52 days, respectively. Two patients were on corticosteroid treatment and blood samples were obtained.

CSF was obtained by lumbar puncture within 2 weeks of the onset of relapse from 10 of the patients with AON and from 17 MS patients during an exacerbation.

Methods

Lymphocyte studies

Peripheral blood lymphocytes. Blood was obtained by venipuncture and immediately mixed by glass beads. 5 ml of defibrinated blood was mixed with an equal volume of physiological saline and carefully layered on 3 ml of Lymphoprep® (Nyegard & Co) in a plastic tube, centrifuged for 40 min at room temperature at exactly 400 g at the interface between blood, saline and Lymphoprep®. The cells from the interface were carefully removed and washed 3 times in hanks balanced salt solution (HBSS) and the concentration adjusted to 4×10^6 ml. Monocyte contamination as judged by latex particle agglutination was usually 5-10%.

ocytes 0.2 ml of the lymphocyte suspension was mixed with 0.2 ml of 0.5% sheep erythrocytes in HBSS. The mixture was incubated at 37°C for 15 min, centrifuged and stored overnight. The supernatant was then removed, and a drop of 0.6% glutaraldehyde added to the cells. After incubation in ice water for 20 min, the cells were resuspended. A drop of the suspension was placed on a glass slide and covered with a coverslip. A count of 200 lymphocytes was counted at a magnification of 600 \times . Lymphocytes with more than 3 sheep erythrocytes were counted as rosette forming cells (Nass & 1978).

lymphocytes Receptors for the Fc portion of immunoglobulin C were used as marker for B indicator cells (EA) were prepared by sensitizing ox erythrocytes (E) with one unit of rabbit IgG antibodies (A). 0.2 ml of the lymphocyte suspension containing 10^6 lymphocytes was mixed with 0.2 ml of indicator cells (EA). The mixture was stirred at room temperature for 15 min, stored at 4°C overnight and counted as for E-rosettes (Christensen et al. 1977).

lymphocytes The total number of lymphocytes/ml was determined in a Fuchs-Rosenthal counting chamber by counting the cells in the first and the last portion of the fluid sample. Count of 0.5 cells/ml were accepted as normal. Enumeration of T lymphocytes in CSF was performed as described by Nass (1976). Briefly, 3 ml of CSF were allowed to drop from the tube into a plastic tube containing 6 ml of HBSS. The tube was centrifuged at 400 \times for 40 min at room temperature. The supernatant was then removed and 0.07 ml of sheep erythrocytes suspended in HBSS to 0.5% were added. Incubation, recentrifugation and the counting steps of the technique were then performed as described above for peripheral blood lymphocytes. Because of the low numbers of lymphocytes present in the CSF from patients with AON, tests for Fc receptor bearing cells in the CSF could not be included. The CSF immunoglobulin C (IgG) were quantitated by single radial immunodiffusion on commercial plates (Behringwerke AG, Marburg, Lahn, West Germany). The normal concentration ranges given for CSF IgG are 8–40 mg/l. The CSF protein concentrations were determined by the modified Folin Ciocalteu method described by Scharkierle & Lollack (1973). The upper normal value for CSF protein is 0.5 g/l. Gel electrophoresis was performed on CSF concentrated $\times 50$ using Amicon concentrator B 10 (Amicon Corp., Lexington, Mass., USA). The samples were inspected for oligoclonal bands in the gamma area.

Statistics Percentages and concentrations are given as mean value \pm standard error of mean. The significance of the data was established by the use of Student's *t* test (two-tailed) for paired examinations by Wilcoxon test for the CSF.

Results

Peripheral blood. The mean lymphocyte numbers in peripheral blood from patients with AON was 2697 cells/ml, slightly higher than in the controls (2092 cells/ml, $P=0.018$), while the MS patients had 2166 cells/ml.

lymphocytes The percentage of T lymphocytes was significantly reduced ($41\pm 9\%$).

Table I

Total and relative T lymphocytes in peripheral blood from patients with acute optic neuritis

Group	No of patients	Lymphocytes ml	T lymphocytes %	Thymus
Acute optic neuritis	10	2697 ± 248 ¹	54 ± 3 ²	11 %
Active multiple sclerosis	21	2166 ± 264	47 ± 3 ³	9 %
Controls	30	2090 ± 114	66 ± 2	13 %

1 = $P = 0.019$ 2 = $P = 0.003$ 3 = $P < 0.0005$ 4 = $P = 0.005$

$P = 0.005$) in patients with AON when compared to the control group (4). However, the absolute numbers were slightly increased compared to the controls. Patients with active MS showed a decrease in both relative and absolute T lymphocytes (Table I).

B lymphocytes Both the relative and absolute numbers of B lymphocytes were slightly increased but not significantly increased in patients with AON. This increase was not marked as that observed in patients with active MS (Table II).

CSF lymphocytes The CSF samples from 2 out of 10 AON patients and 11 of 21 MS patients had a lymphocytic pleocytosis. The CSF T lymphocyte percentages could be determined in 4 AON patients and showed a significant increase (mean 88.8%) as compared to 78.0% in the control group (Table III). All MS patients had increased percentages of CSF T lymphocytes ranging from 89.06% (mean 94.1%).

Table II

Total and relative numbers of B lymphocytes in peripheral blood from patients with acute optic neuritis

Group	No of patients	B Lymphocytes %	B lymphocytes ml
Acute optic neuritis	10	39 ± 3	94 ± 13 ¹
Active multiple sclerosis	21	39 ± 3 ²	108 ± 7
Controls	30	36 ± 2	540 ± 43

1 = $P < 0.005$ 2 = $P = 0.003$

Table III

T lymphocytes in CSF from patients with AON and MS

Group	No of patients	T lymphocytes % mean (range)
Acute optic neuritis	4	88.8 (87.9-92)*
Active multiple sclerosis	17	99.8 (89-96)
Controls	17	78.0 (55-89)

* $P = 0.002$ (Wilcoxon test) ** $P < 0.0005$ (Wilcoxon test)

patients. In the 10 AON patients the total protein concentration was mean 0.51 g/l (ranges 0.39-0.96). The mean IgG concentration was 4.2 mg/l (ranges 2.7-7.9). The relative IgG concentrations were elevated above the upper normal value of 0.15 in 4 patients. Two patients had oligoclonal IgG bands in agarose gel electrophoresis.

In the 17 MS patients the total protein concentration was mean 0.75 g/l (ranges 0.5-1.1). Sixteen of the patients had a relative increase in the relative IgG concentrations. The mean IgG concentration was 10.7 mg/l (ranges 5.0-23). Ten of the 12 patients examined with agarose gel electrophoresis had oligoclonal bands.

DISCUSSION

The present study compares the immunological findings in a group of patients with AON to that of a group of patients with active MS.

AON patients had a significant increase in peripheral blood lymphocytes as compared to controls. The relative concentration of T lymphocytes decreased when the absolute numbers were normal. This finding is different from that in MS patients who have a significant decrease of both absolute and relative T lymphocyte concentrations (Næss & Nyland 1978). Recently it has further been shown that a decrease of T lymphocyte subsets, the T suppressor cells, also are decreased in active MS (Oldstone & Oldstone 1979). These T cells have a suppressor activity on the B cell proliferation and differentiation *in vitro*. We found slightly but not significantly increased numbers of circulating B lymphocytes in patients with AON. This may suggest that humoral immunity is operative in the disease. However, no values have been reported for the serum concentration of immunoglobulins (Oldstone et al 1973). Abnormally high titres of measles antibodies have been found in CSF from AON patients but the titres are lower than in patients with MS (Perkin

The CSF samples from 2 AON patients (20%) and from 14 MS patients had a mononuclear pleocytosis. Similar results have been reported (Sandberg & Bynke 1973; Perkin 1979). The T lymphocyte percentage was normal in AON (mean 92.8%) Traugott (1978) found a mean of 72% CSF T lymphocytes in 2 patients with AON compared to 80% in relapsing MS. Normal values for lymphocytes were not given but a control group of patients with neurological diseases had T cell percentage of 65 in CSF. In another study, Kam Hansen et al (1978) found 79.9% T cells in CSF from five AON patients compared to 88% in MS CSF. This similarity in lymphocyte pattern in AON and MS patients is further reflected in a recent cytologic examination (Taskinen et al 1979) showing an identical abnormal cellular pattern in both conditions. The CSF lymphocytes form part of the total pool of circulating lymphocytes and the increased proportion of T lymphocytes may reflect selective crossing of the blood liquor barrier by the T cells.

The CSF relative IgG concentration was above normal in 4 of 10 patients with AON (40%) while 16 of the 17 MS patients had elevated concentrations. The same differences were observed between the two groups of patients when analysed by agarose gel electrophoresis. Oligoclonal bands were present in CSF from 100% of the AON patients and 83% of the MS patients. Our results correspond well with results published by Sandberg & Bynke (1973). The antibody specific oligoclonal IgG has not been determined but a small proportion seems to be measles antibodies (Link et al 1973). The occurrence of oligoclonal CSF in patients with AON does not invariably predict a later development of MS (Sandberg & Bynke 1973).

In conclusion, the limited work done on lymphocyte subpopulations indicates changes in blood and CSF similar to those observed in patients with MS. The demyelinating lesions are also similar but the degree of involvement is more limited in patients with AON without additional clinical signs. This is reflected in the lower incidence of elevated relative IgG concentration and oligoclonal IgG bands in CSF from AON patients.

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A STUDY IN THE MEASUREMENT OF CORNEAL ENDOTHELIAL CELL DENSITY USING THE SPECULAR MICROSCOPE

BY

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Six subjects who never wore contact lenses underwent six determinations of corneal cell density using each of two measurement modes. Mode A there was applanated once and pairs of photographs were taken. Mode B there was applanated and a single photograph was taken. For each measurement mode data were collected on two separate sessions (Test, Retest). Chi-square analyses revealed a significantly larger proportion of uncountable photographs from the first measurement session and analysis of variance revealed that Mode A was less reliable than Mode B upon retesting. It was concluded that Mode B was the preferred measurement mode and that many more than six determinations be made on the initial session in order to obtain six readable photographs. Finally estimates of inter and intra session variation, inter-observer variation and inter and intra-observer variation are given.

Key words: cornea - corneal endothelial cell density - specular microscope human cornea

Determination of endothelial cell density using the specular microscope has been increasingly valuable in the study of effects of various medications and surgical procedures on the structure and function of the corneal endothelium (Binkhorst et al 1977 Hirst et al 1977). A variety of problems have been encountered in the assessment of endothelial cell density using current techniques and the need for evaluating sources of error has been emphasized by many investigators (Laule et al 1978 Binder 1978 Sperling & Gundersen 1979).

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purpose of the present study is to identify and quantify sources of variability in measurement of endothelial cell density. On the basis of these findings, conclusions are made regarding the utilization of specular microscopy, especially as it relates to longitudinal prospective studies.

Material and Methods

Endothelial cell density (cells/mm²) was measured on a randomly selected subset of six subjects (two females, four males). All subjects had clinically normal corneas, never wore contact lenses and had no history of ocular disease. Two subjects had the right eye measured and the other three subjects had the left eye measured.

A Syber specular microscope was used to visualize the corneal endothelium. Six micrographs were taken of the central corneal endothelium using the photographic attachment according to the method suggested with the instrument (Syber 1972). Six specular micrographs of the study eye were obtained by the ophthalmologist (E. M.) using each of two measurement modes (Mode A and Mode B). For Mode A the photographs were taken pairwise, i.e. the eye was applanated once and a pair of photographs was taken. For Mode B the photographs were taken independently, i.e. the eye was applanated before each photograph was taken and then the dipping cone was removed from the corneal surface. For each measurement mode data were collected on two separate sessions (Test/Retest) which were three days apart. For each mode and each session the order in which the eyes were measured was randomized.

A black and white film processing technique was used to produce non-identifiable proofs. Endothelial cells were counted by a single technician using the fixed frame method (Fuchs & Kaufman 1976). A counting overlay of 0.04 mm × 0.3 mm was chosen. This dimension yielded the largest proportion of cells relative to the proof size in which could easily be counted. Proofs were graded as Good (countable, clearly distinguishable), Fair (countable but some cells not clear), Bad (not countable). For some (approximately 10%) of the Fair proofs a smaller counting overlay (0.04 mm × 0.2 mm) was used.

To estimate intra-observer variation, a subset of photographs was counted twice by the same technician. This same subset was also counted by a second technician for the purpose of assessing inter-observer variation. Both technicians utilized the same counting procedures.

The resulting data were analyzed statistically using chi-square analysis with continuity correction for trend (Maxwell 1971) and analysis of variance with repeated measures (Niffler & Azen 1979).

Results

Table I summarizes the distribution of the readability (Good Fair Bad) of photographs per subject. The proportion of Bad photographs was larger for the first session (Mode A Test) than that for each of the other sessions ($\chi^2 = 13.1$ $P < 0.005$). There was no significant difference in proportions of Bad photographs for sessions 2-4 ($\chi^2 = 0.5$ NS). For photographs were randomly distributed throughout the measurement period.

Table II summarizes for each subject and session the mean (\pm SEM) index of the Good and Fair photographs. Also shown in the table is the mean density for each subject (averaged over sessions). Analysis of variance (F) showed a considerable (although non significant) mean difference (11 mm^2) between Test and Retest for Mode A and a negligible mean difference (-11 cells/mm^2) for Mode B.

Table IV presents for each mode estimates of intra and inter session standard deviations. Modes A and B were equally variable within a session ($F = 1.5$ NS). Mode B was significantly less variable than Mode A upon retesting ($F = 5.06$ $P < 0.05$). Also shown is the intra pair standard deviation for Mode A i.e. the average pairs of photographs for those pairs that were graded Fair or Good. Inter cell density within pairs using Mode A was equivalent to that with a retest using Mode B ($F = 1.07$ NS).

Sixteen selected photos were counted twice by the same technician on separate occasions. The mean difference in cell density was 5 cells/mm^2 (NS). The same set of photos was counted by a second technician. The difference in cell density was 117 cells/mm^2 ($t = 3.26$ $P < 0.01$).

Table I
Summary of the Readability of Photographs by Session

Readability* of photographs per subject	Session 1 Mode A (Test)	Session 2 Mode B (Test)	Session 3 Mode A (Retest)	Session 4 Mode B (Retest)
Good	3	16	7	6
Fair	20	17	24	3
Bad	13	3	5	3
Proportion of Bad photographs	0.36	0.09	0.14	0.09

* Good = cells clearly distinguishable. Fair = some cells not distinguishable. Bad = uncountable.

Table II

Endothelial Cell Density (cells/mm²) Summary Statistics ($\bar{x} \pm \text{SEM}$ (n))

Session 1 Test (Mode A)	Session 2 Test (Mode B)	Session 3 Retest (Mode A)	Session 4 Retest (Mode B)	Average (weighted by n)
988 ^o 2 \pm 93.5 (5)	3082.1 \pm 107.5 (6)	3193.9 \pm 41.2 (6)	3193.8 \pm 79.1 (4)	3074.9 \pm 46.9 (21)
963.3 \pm 67.2 (6)	2709.6 \pm 110.3 (4)	2948.8 \pm 72.6 (5)	2799.1 \pm 62.5 (5)	2817.0 \pm 43.4 (90)
985.0 \pm 67.5 (4)	9957.9 \pm 35.6 (6)	3061.7 \pm 52.6 (6)	9839.6 \pm 85.1 (6)	9934.7 \pm 34.5 (92)
248.9 \pm 48.1 (3)	9818.3 \pm 54.4 (6)	2948.8 \pm 33.3 (5)	9679.5 \pm 50.0 (6)	2798.9 \pm 98.0 (20)
2471.2 \pm 73.5 (3)	2415.7 \pm 64.5 (6)	2415.7 \pm 45.6 (5)	9457.4 \pm 51.5 (6)	2436.5 \pm 33.0 (90)
9374.1 \pm 125.0 (2)	9349.1 \pm 103.3 (5)	2353.2 \pm 85.9 (4)	2415.7 \pm 60.8 (6)	9376.5 \pm 51.3 (17)

Discussion

data from this study indicate that the measurement of endothelial cell density using the specular microscope and measurement Mode B produces reliable data upon retesting in normal subjects (Table III). Mode A, on the other hand, is shown to be somewhat less reliable than Mode B upon retesting (Table III). Although the intra-session variation was statistically equivalent to that using Mode B (Table IV), the non-significant reduction in variation within pairs of measurements compared to variation within replicates (167 cells/mm² vs. 173 cells/mm², Table IV) may be attributed to slight movement in the cornea relative to the applanating device during the recharging of the flash attachment to the Nikon photographic camera (average time for recharge = 5 seconds). The significantly larger number of unreadable photographs for the first session, but not in the third session (both Mode A), suggests that there may be a learning effect on the part of the subjects. The subjects did, in fact, exhibit some uneasiness during the initial measurement session. We can only speculate that if Mode B had been the initial method used, a similar pattern would have occurred.

During the developmental stages of a proposal to study the effects of intra-ocular pressure on the corneal endothelium utilizing specular microscopy, we devised the present study to determine which methods would be appropriate for long-term

prospective studies. The results of this study suggest that in longitudinal studies: a) more than six specular micrographs be taken during the first flow for each order to obtain quality photographs; b) that the replicate measurement (Mode B) be utilized; and c) the same observer be used to count the results. That these normal subjects had no change in cell density over the period of study, variations in cell density of $\pm 6\%$ are within the limits of measurement. Since recounting the same proofs by a different technician may vary on the order of $\pm 4\%$, the same technician should be used in follow up evaluations if possible.

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Table III
Analysis of Variance of Endothelial Cell Density (cells/mm²)

Mode	Average Cell Density		F	P
	Test	Retest		
A	2682.1	2800.2	5.59	<0.05
B	2730.3	2719.2	0.09	

Table IV
Precision of Cell Density Measurements (cells/mm²)

Standard Deviation (5% CV)*	Mode A	Mode B
Intra session	139 (2%)	113 (6%)
Intra session	135 (5%)	60 (9%)
Intra pair	167 (6%)	-

* The coefficient of variation CV = $\frac{sd}{\bar{x}}$ where sd is the standard deviation and \bar{x} is the mean cell density = 2750 cells/mm².

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CORNEAL THICKNESS AND ENDOTHELIAL DAMAGE AFTER INTRACAPSULAR CATARACT EXTRACTION

BY

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In a prospective study corneal thickness and specular microscopic findings of corneal endothelium are reported in 37 patients undergoing intracapsular cataract extraction. Central endothelial cell loss was estimated six months after the operation and was found to correlate significantly to the immediate post-operative increase in central corneal thickness. A subgroup of patients showing slight endothelial dystrophy prior to the operation showed a significantly higher increase in corneal thickness fourth day after the operation. Six months after the operation a significant residual increase in corneal thickness was found for this group while the rest of the patients had returned to their pre-operative levels. No correlation was found between cell loss and residual corneal thickness increase at this time. Six months after the operation a vertical difference in cell density was found. This difference could be correlated to the age of the patient presumably indicating a less complete redistribution of the cell population in older patients.

Key words: cataract extraction — cell loss — corneal thickness — endothelium specular microscopy

The damage exerted on the corneal endothelium during cataract extraction has been the subject of several specular microscopic studies (Bourne & Kaufman 1977, Cheng et al 1977, Forstot et al 1977, Hirst et al 1977, Rao et al 1977, Waltman 1978, Rao et al 1979, Gahn et al 1979, Abbott & Foster 1979). A working hypothesis has been that the amount of the endothelial trauma, i.e. cell loss is somehow related to the function of the endothelium and therefore to its hydration. The information yielded by the endothelial reflex on this respect is however still unsettled.

post-operative endothelial cell density varies with time and location on the cornea. Evidence is now accumulating that the gradual decrease in cell density seen a few days after the operation (Hirst et al 1977 Rao et al 1978 Galin et al 1979) is due to a redistribution of the cell population (Rao et al 1978 Sugar 1979) leading with time to even out imbalances in cell density created during surgery. This implies that a lowered central cell density is an inaccurate index of total cell loss in the early phases after the operation. The present study reports the immediate effect of endothelial damage on corneal thickness following intracapsular cataract extraction. In order to improve estimation of the cell loss post-operative cell densities were not estimated until six months after surgery.

Subjects and Methods

A total of 40 subjects with senile cataract entered the study. This group comprised all patients newly admitted to the eye department during a two months period where indication for intracapsular cataract extraction was found. Patients with co-existing eye diseases such as glaucoma, uveitis or corneal diseases were excluded. Generally patients were not included if endothelial dystrophy was revealed in routine slit lamp examination. Not seldomly, however, the specular microscopic examination revealed a guttate endothelium in the central reflex which was not readily detected in ordinary slit lamp examination. In such cases surgery was not abandoned and the patients were included in the study. The surgical procedure was intracapsular cryoextraction with corneal incision and irrigation. For irrigation a Ringer solution was used. The wound was closed with running 10-0 nylon suture. The operations were performed by a number of surgeons. No regard was paid to the particular surgeons experience. Occurrence of vitreous loss or other pre-operative complications did not exclude the patient from the series. Prior to surgery the central endothelium of all patients were photographed with a contact specular microscope (Olsson 1979). The central corneal thickness was measured the day before and on each of four days after surgery using a modified Haag Streit pachometer (Ehlers & Sperling 1977). All measurements were single determinations taken as close as possible to the 5 μ position on the scale reading of the pachometer and were done by the author daily after noon. The standard error of single determinations with the present method has previously been found to be 5-6 μ (about 1%) estimated from a large (>90) number of measurements of several subjects. Six months after the operation the patients were asked to attend the clinic for re-examination. The central endothelium was photographed as described above and in addition an area 3 mm superiorly in the center of the cornea was photographed by directing the gaze of the contralateral eye. Again corneal thickness was measured. Intraocular pressure was measured with an applanation tonometer attached to the slit lamp. Seven patients did not show up for re-examination. One patient was excluded because of post-operative glaucoma. Age range of the remaining patients, 16 men and 21 women, was 57-87 years with a mean of 73.6 years. These 37 patients are reported on in the following. Unless specified otherwise statistical analyses were based on distributional methods. For comparison of means Student's *t* test was employed.

Results

Pre operative and post operative central cell density of operated eye was (SD) 2541 (\pm 423) and 1894 (\pm 501) cells/mm² respectively with a mean of 25.1 (\pm 16.4) %. Pre-operative central corneal thickness of operated eye was (\pm 0.031) mm. No correlation was found to pre-operative cell density ($r = 0.41$, $P = 0.3$). The immediate increase in central corneal thickness was 0.083 (\pm 0.007) (\pm 0.050), 0.059 (\pm 0.027) and 0.054 (\pm 0.028) mm the first, second, third and fourth day after the operation respectively. For each day this increase was found to correlate significantly to the central cell loss ($r = 0.73$, 0.59 , 0.56 and 0.51 , $P < 0.001$ for day one through day four respectively). The strongest correlation was thus found on the first post-operative day (Fig. 1). Dotted line in Fig. 1 indicates regression line y on x calculated using the method of least squares for the increases below 250 μ . Intersection on ordinate is not significantly different from the origin.

Six of the patients turned out to have guttae in the endothelium as revealed by the pre operative photographs of the central endothelium. These changes

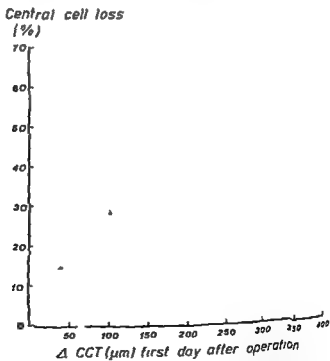


Fig. 1

Correlation between first day post-operative increase in central corneal thickness (Δ CCT) and central endothelial cell loss estimated six months after cataract extraction. The dotted line indicates regression line y on x ($r = 0.73$, $P < 0.001$).

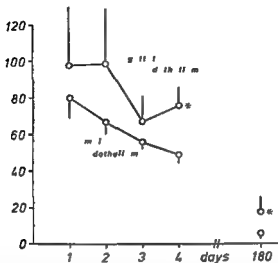
Δ CCT (μ m)

Fig 2

post-operative increase in central corneal thickness (CCT) in 31 patients with normal endothelium and 6 patients with minor dystrophic changes in the endothelium. Asterisks indicate significant ($P < 0.05$ by Student's *t* test) difference among the groups on day four. Persistent elevated thickness six months after surgery for the group with guttate endothelium. Vertical bars indicate standard errors of the mean.

teral. Mean pre-operative corneal thickness of this group was $0.540 (\pm 0.017)$ mm, not significantly different from the rest of the patients. The post-operative increase in corneal thickness for this group was larger than in the patients with normal endothelial reflex and became significantly different fourth day after the operation (Fig 2). The mean cell loss of $29.7 (\pm 20.1)\%$ for this group was however not significantly different from the rest of the patients.

For the total group neither pre-operative cell density, visible pleomorphism of cells, pre-operative corneal thickness, age nor sex was significantly related to the thickness increase or central cell loss.

Six months after the operation, central corneal thickness was almost normalized in the normal group. Mean residual thickness increase was $0.006 (\pm 0.018)$ mm, not significantly different from zero. In the group of pre-operative endothelial guttae, the residual thickness increase was $0.018 (\pm 0.020)$ mm, which is close to but significantly ($P < 0.05$) different from zero. In these patients, the endothelial changes had worsened in all cases; that is, the defects in the endothelial reflex were larger and more numerous than before the operation (Fig 3). No guttae-like changes were found in those eyes with previous normal endothelial reflex (Fig 4).

No correlation was found between cell loss and residual corneal thickness 1 time in the entire group ($r = 0.22$ $P > 0.1$). All corneas were clear. The thickness of unoperated eye showed a mean change of -0.001 (± 0.004) -0.003 (± 0.012) mm in the normal and guttae group respectively.

The superior counts found six months after the operation were as a group, that the central counts. Mean decrease from central to superior counts was range -3 to $+57\%$. This vertical difference in cell density was found to be significantly to the age of the patient (Fig. 3). For graphic simplicity the data have been pooled and averaged in three age groups. No difference was found in the vertical density difference in the guttae group as compared in the normal group (mean values 26.5 (± 18.5) % $n = 3$ vs 18.0 (± 16.4) % $n = 29$ respectively $P < 0.4$). In five patients counts from the superior region were not available due to poor quality of photographs or extensive guttae-changes. No relation was found between the vertical density difference and residual corneal thickness 6 months after surgery.



Fig. 3

Central corneal endothelium before (top) and six months after (bottom) cataract extraction in a seventy-one year-old woman with guttate endothelium. Cell density has decreased 49% from 2480 to 1270 cells mm^2 . Central corneal thickness was 0.190 mm first day after the operation. Bar = 100 μ .

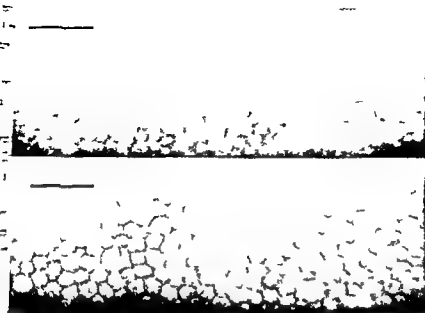


Fig 4

central corneal endothelium before (top) and six months after (bottom) intracapsular cataract extraction in a sixty nine year-old woman. The cell density has decreased 3% from 1820 to 1720 cells mm^2 . Central corneal thickness increased 0.190 mm first day after the operation. Bar = 100 μ .

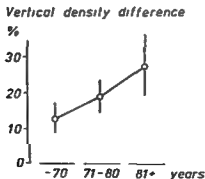


Fig 5

mean difference in cell counts from the central endothelium and 3 mm superiorly found six months after intracapsular extraction. The vertical density difference is significantly correlated to the age of the patient. (Spearman's rank correlation coefficient = 0.59 $P < 0.05$) Bars indicate standard errors of the mean.

Discussion

The mean cell loss reported after intracapsular cataract extraction ranges from 6-21% (Bourne & Kaufman 1976 Cheng et al 1977 Forstot et al 1977 Rao et al 1978 Drews & Waltman 1978 Galin et al 1979 Abbon & 1979). In these studies the post-operative time until evaluation generally ranges from a few days to several months. The mean cell loss of 20% found six months after the operation in the present study may seem higher than in the studies above. Some of this discrepancy undoubtedly is accounted for by the long post-operative period in the present study. Because of the sliding phenomenon central counts may be expected to indicate total cell loss better than the long post-operative follow up period. Galin et al (1979) state that the post-operative decrease in central counts is minimal after three months. The still present cell density difference found in the present study after six months (Fig 5) indicates a complete redistribution of the cell population has not taken place at this time at least in the age range studied. Whether the central count after this time reflects the mean decrease in cell density is unclear. To answer this a complete mapping of the corneal endothelium post-operatively seems necessary.

Another factor which must be considered when comparing cell losses reported by different investigators is that only clear corneas can be studied. At the post-operative follow up examination all corneas were clear ensuring that no endothelial pathology was excluded on the grounds of an oedematous cornea, a problem which may occur in the early post-operative days and especially in case of great cell loss.

The average increase in corneal thickness found in the present study in the early post-operative days seems to be in agreement with the results of Norn (1973) & Maumenee (1975) and Bramsen et al (1978). Somewhat greater than the increase was found by Cheng et al (1977). Giardini & Cambiaggi (1965) in a study of extra- and intra-capsular extractions found an average of 40% increase in corneal thickness on third day after the operation. Presumably this high increase may be due to the different surgical technique employed twenty years ago (Giardini 1973).

Most authors agree that the corneal thickness returns to normal less than six months after the operation (Cheng et al 1977 Norn 1973 Wood & Maumenee 1975) even in the case of very high immediate increase (Giardini & Cambiaggi 1965). At variance is the finding of Miller & Dohlman (1970) who stated that of 50 unilateral aphakic subjects more than six months after the operation showed increased corneal thickness on the operated side. As was shown by Wood & Maumenee (1975) and as was the tendency in the present investigation the ultimate thickness depends on the pre-operative condition of the endothelium.

ation was given on the status of the endothelium in the series reported by & Dohlman (1970). The present investigation revealed a strong correlation between the immediate post-operative central cell loss and the central corneal thickness. Like the situation with the total volume change or water intake of the cornea, the central increase in thickness is dependent on the distribution within the cornea, which varies considerably with time and among different individuals (Giardini & Cambiaggi 1956). Generally however the cornea is thicker in the upper part and thinner in the lower part with an intermediate thickness in the center. By expressing the cell loss as per cent of initial value the relationship depicted in Fig. 1 is in fact a correlation between an index of the water content of the cornea and an index of the area broken down of the endothelium as a result of the surgery. This relationship bears a remarkable resemblance to the situation in acute glaucoma (Olsen 1980).

The reason why other authors who have measured both the corneal thickness and the central cell loss did not find this close relationship may be found in the shorter post-operative period studied. Bourne & Kaufman (1976) noted however that those corneas with significant cell loss also had greater increase in thickness. Laing et al. (1977) noted that cataract extraction with lens implantation gave more cell loss and greater increase in corneal thickness than without lens implantation. Experimentally the quantitative correlation between damaged endothelial area and corneal thickness response has been demonstrated by Honegger (1962) and Van der Velden et al. (1977).

It is noteworthy that the cell loss observed in the present study did not induce a permanent decrease in the deturgescence function of the cornea. Only those with pre-operative guttate endothelium had not returned to pre-operative corneal thickness after six months. It appears that the integrity of the normal endothelium, although deprived up to 63% of its cells (found in the present study) alone can maintain normal corneal thickness at least in the range of lowered cell densities observed in the present study. A similar observation has been made by Forstot et al. (1977) and in corneal grafts (Bourne & Kaufman 1976a, Laing et al. 1976, Sato et al. 1978). Another remarkable result was that no newly formed guttae had developed as a result of the cell loss. This challenges the hypothesis held by Capella (1979) that the cell damage as such initiates the production of abnormal Descemet's membrane material and add some evidence against the view of Fuchs' endothelial dystrophy being a low cell density syndrome (Olsen 1980).

It was surprising to find a persistent vertical difference in cell density six months after surgery. Whether this imbalance in cell density was a stable situation or a further reduction would occur with time is yet unclear. Apparently the mobility of

the cells is dependent on the age of the patient as shown in Fig 3. The functional significance of these findings remains unknown. It is hardly doubtful however if the cells were not able to move there was no way by which the endothelium could reestablish an intact barrier function in response to injury.

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arent adhesive tape which could be removed temporarily during measure

gas in each tank was analysed by a specialised laboratory and claimed to be within 0.1%. Two oxygen mixtures were used: 2.1% oxygen and 3.15% corresponding to 16 and 24 mmHg.

In view of the results of Polse & Mandell (1970) who found no significant difference between dry or humidified gas on corneal hydration, we used only dry gas directly from the storage cylinders.

Corneal sensitivity measurements

Corneal sensitivity was measured using the Cochet-Bonnet (1960) aesthesiometer based on a filament divided by Boberg & Ans (1955). The instrument consists of a nylon monofilament of 0.12 mm diameter that can produce pressures ranging from 11 to 200 mg/0.0115 cm². The aesthesiometer was mounted in a holder so that it could be moved in the x, y and z directions by means of three knobs. Thus, it was possible to achieve reliability in stimulation of the corneal point: a steady speed of application and a perpendicular corneal contact. A point near the lower limbus was stimulated and the slightest bend of the nylon wire was defined as corneal contact.

During measurements, the subjects fixated a target directly in front and considerably above the normal line of sight. A peripheral corneal point was chosen so as to reduce apprehension (Bonnet & Millodot 1965).

Measurements of corneal touch threshold (CTT) were made subjectively (Millodot 1973). The experiment began with the lowest pressure and continued in an ascending fashion. At

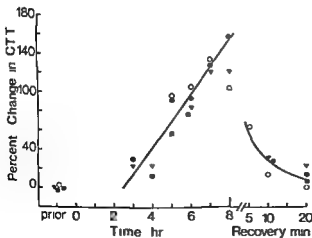


Fig. 1

Corneal Touch Threshold as a function of time with exposure to 2.1% oxygen and recovery: ● subject A, ▼ subject B, ■ subject C.

each predetermined length of the monofilament (with increments equal to 0.5 cm). The contacts were made with at least one blank to test the subject's reliability. The indicated when he or she felt the probe by operating a buzzer. From these results, CTT was defined as the length of the monofilament at which the subject responded to the number of stimulations. This length was converted into pressure using a pre-calibrated curve for the instrument. All measurements were taken when the humidity in the room ranged between 50% and 60% because the nylon monofilament is affected by humidity. The accuracy of this measurement is usually within $\pm 5\%$.

Procedure

Both eyes of the subject were tested for corneal sensitivity in the morning before the experiment. The subject had been awake for at least two h in order to minimize the diurnal effect (Mandell & Fatt 1965; Hirji & Larke 1978; Millodot 1979). The subject wore air tight goggles (with the adhesive tape) were fitted firmly on the subject's face. The gas was then turned on. Measurements of corneal sensitivity were made before and after three h by removing the transparent adhesive tape and increasing the flow of gas. The procedure took between 40 and 55 seconds and the adhesive tape was immediately stuck back on the goggle. Following the experimental session, measurements were again collected for up to 40 min to assess recovery. All data were collected from three subjects because of the constraints produced by keeping the subject connected to a gas tank through the goggles for so many h. All measurements were repeated on one subject a week later.

Results

Fig. 1 shows the increase in CTT with 2.1% O_2 for the three subjects and the results of a session on one of them as compared to the value obtained prior to the experiment. After about three h CTT rises rapidly and linearly reaching a 130% increase at the end of the experiment (8 h). Recovery is quite fast and most of corneal sensitivity had returned within 20 min. The results are similar with 3.15% O_2 as illustrated in Fig. 2. However it takes longer (about 4 h) before CTT begins to rise. Afterwards the increase is very rapid reaching 100% at the end of the experiment (10 h). With both oxygen mixtures there is a rapid recovery providing further proof that sensitivity had indeed diminished during the experiment.

The latency of several h before any appreciable loss of corneal sensitivity was observed explains why Polse (1978) did not find any change in sensitivity in his work since he carried out his measurements for a period of only 2.5 h.

Measurements on the control eye showed no appreciable difference between the pre-experimental session indicating that the goggles had no effect on the cornea.

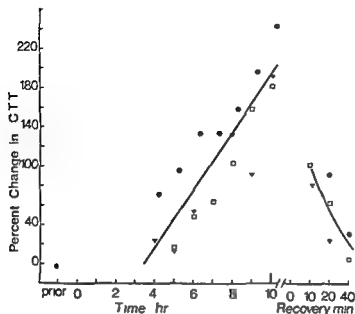


Fig 2

al Touch Threshold as a function of time with exposure to 31% oxygen and recovery: ● subject A ▼ subject B

Comment

Results of this investigation show that exposing the cornea to a partial pressure of oxygen of 21% (or 16 mmHg) and 31.5% (24 mmHg) lead to a progressive and rapid increase in corneal touch threshold.

At a partial oxygen pressure of 16 mmHg it took nearly 6 h to observe an increase in CTT (or loss of sensitivity) of 100%. Whereas it took some 7 h with a pressure of 24 mmHg (see Fig. 3). In another study by Millodot & O'Leary (1979) it was found that when the eyes are closed—that is the oxygen pressure at the corneal surface is 33 mmHg—an equal increase in CTT occurs after 15 h. This result is also shown in Fig. 3. And with the wear of hard contact lenses (PMMA) Millodot (1976) showed that after 10 h CTT was increased by 100%. According to Farris et al. (1979) the oxygen deprivation induced by wearing hard contact lenses is greater than that caused by lid closure and from the data of Fatt & Hill (1970) it can be estimated after taking into account the effect of blinking to average around 38%. Inserting this value in Fig. 3 shows that there is a clear relationship between

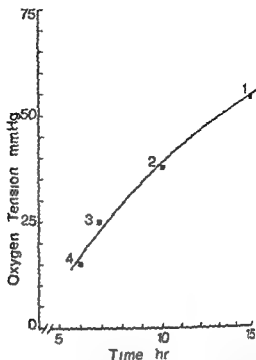


Fig 3

Oxygen tension at the corneal surface and time to produce a 100% loss in corneal sensitivity
 1 eyelid closure (Millodot and O'Leary 1979) 2 hard contact lenses (Millodot & O'Leary 1979)
 3 15% oxygen mixture 4 21% oxygen mixture

the loss of corneal sensitivity and the partial oxygen pressure at the corneal surface. Thus it appears unequivocal that corneal sensitivity diminishes markedly when oxygen tension at the corneal surface is below normal. This conclusion agrees with the fact that after permanent wear of contact lenses with low oxygen transmission corneal sensitivity is reduced by 100% after 16 weeks (Lark & 1979).

Since these alterations occur at oxygen tensions well above that at which the cornea starts swelling (Polse & Mandell 1970) it would be inappropriate to assume that corneal thickness measurements are satisfactory indicators of a normal state. At present it seems that corneal touch threshold measurements give a good indication of disturbed corneal physiology and it is suggested that this factor should be worth monitoring clinically.

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THE FATE OF PRESERVED AND TRANSPLANTED HUMAN CORNEAL ENDOTHELIUM

BY

PEKKA RUUSUVAARA

This paper evaluates the numbers of corneal endothelial cells that survive transplantation. The donor endothelium was photographed with a specular microscope both before enucleation and in situ after keratoplasty. The cell density was measured in 16 corneas from donors with choroidal melanoma. Of these donor corneas 12 were cryopreserved, two were preserved in McCall and Kaufman's (MCK) medium and two were transplanted fresh. The average follow-up period after keratoplasty was 11 months.

The mean endothelial cell loss for the whole series was found to be 49%. The mean cell loss for the cryopreserved corneas was 53%. In the four other recipients with donor corneas that had been stored in MCK medium or transplanted fresh the mean cell loss was 32%.

The corneas preserved in MCK medium had the highest cell densities in the transplants with cell losses of 21 and 22%. Cell losses in the two corneas transplanted fresh were 40 and 44%. Cell losses in the cryopreserved grafts had a wide variation 33-77%. No correlation could be found between cell loss and either the age of the donor or the duration of preservation.

Freezing and thawing was found to damage a proportion of the cells in that they did not stain with para-nitro-blue tetrazolium (p-NBT) after preservation. Transmission and scanning electron microscopy also revealed changes in the intercellular spaces and some cell disruption in cryopreserved grafts.

Key words: corneal graft endothelium - endothelial ultrastructure - corneal preservation - specular microscopy - cell viability - cryobiology

Clinically good results have been reported in penetrating keratoplasties with corneas preserved by several different methods. Measurements of graft endothelial cell densities postoperatively with the specular microscope have established the viability of donor endothelial cells after preservation of whole eyes (1-7).

ber and of excised corneas in M K medium and by freezing (Bourne & nan 1976b Ruusuvaara 1979)

ording to earlier reports (Bourne & Kaufman 1976a Laing et al 1976b et al 1978) endothelial cell density decreases with age (cell size increasing orthonally) The difference in cell density between the right and left eye of the patients is so small that study of the cell population of one eye gives a result enough to represent the cell population of the fellow eye although there is variation in cell density between different individuals Previously it has not possible to calculate exactly how many cells/mm² are lost in each keratoplasty as there was no way of knowing the exact preoperative cell density of the or in vivo With the advent of the specular microscope (Maurice 1968) the tion has completely changed

the purpose of this study was to evaluate the exact numbers of cells lost during procedures of cryopreservation or storage in M K medium and during ipulation of the corneal button in transplantation The corneas were examined a contact specular microscope Because of the difficulty of photographing the eal endothelium of enucleated eyes I photographed the endothelia first in the of the donors with choroidal melanoma before enucleation and later in situ in recipients after keratoplasty This procedure permits direct determination of proportion of endothelial cells that have survived transplantation

sing the corneoscleral rim left after removal of the corneal button (Ruusuvaara 9) after cryopreservation and storage in M K medium I also compared the amicroscopic changes found with the results of vital staining of the endothelia with the cell density in specular microscopy

Material and Methods

Donors and recipients

Donors were 16 patients with malignant choroidal melanoma In nine cases the e endothelium was photographed in vivo before enucleation of the eye and in after keratoplasty In seven cases the endothelium of the donor's fellow eye photographed within a few months after enucleation of the melanomatous eye comparison with that of the graft

The exact endothelial cell loss during preservation and manipulation in kera lasty was calculated from the difference in endothelial cell density between the or cornea before enucleation (or the donor's healthy fellow eye) and the graft or keratoplasty

The recipients ranged in age from 17 to 64 years and the donors from 27 to 72 rs The proportion of men to women among the recipients was 11 to 5

Ten patients had keratoconus one had phlyctenular keratitis and two had pyogenic keratitis. One had an alkali burn of the cornea and two had corneal ulcers of unknown origin. In five cases the corneal beds of the recipients were prepared. Only one patient was aphakic before keratoplasty. Eleven of the 16 patients received grafts with 0-1 HLA mismatches and only five had 2 HLA mismatches; the role of histocompatibility in this series seems to be quite small. All the grafts were cryopreserved and used for keratoconus patients. The average follow-up period was 11 months with a deviation of ± 9 months.

Type of preservation of the donor cornea

In 12 cases the corneas were cryopreserved (Capella et al. 1963) with a scleral rim. The cryopreservation time varied from 1 day to 3 years with an average of 10 months. Two corneas were transplanted after preservation in M & K medium (McCarey & Kaufman 1974) for 1 and 3 days. Two corneas were transplanted fresh. The cadaver time for all these corneas was minimal because all were enucleated from living patients.

Donor button excision and operative technique

Those corneas that were to be cryopreserved or preserved in M & K medium were cut with a large scleral rim and excision from the globe was completed with scissors (Stark 1975). After thawing the graft was punched from the endothelial side as reported in an earlier paper (Ruusuvaara 1979). In two cases the graft was excised directly from the epithelial side of the cornea with the trephine in such a way that only a 7 mm graft was left for immediate transplantation.

All keratoplasties were performed by two surgeons (S. K. H.). The operative technique was the same and 10-0 monofilament as continuous or single sutures was used.

Micrography of the endothelium

The endothelia in the centres of the donor corneas and of the grafts were photographed through a modified clinical specular microscope (Bourne & Kaufman 1961). In each photograph (magnified $\times 500$) the area counted was a rectangle of 0.01 mm² and 25 cells wholly inside this rectangle and those cut by two adjacent sides were counted. Each rim was counted in the four possible different ways and the average was taken as the final value. To obtain a reliable result 3-5 different fields of view were counted.

Vital staining

After removal of the corneal button each corneoscleral rim which had been cryopreserved or preserved in M & K medium was stained histochemically. Staining was done with pararosaniline tetrazolium (p-NBT) which causes precipitation of the dye in the cytoplasm of the endothelial cell if the enzymes are still functioning (Robbins et al. 1963). The prepared corneal endothelium was then examined for viable (stained) cells and non-viable (unstained) cells. The rims of the corneas stored in M & K medium were frozen with liquid nitrogen and thawed before staining and the cryopreserved material was stained after thawing.

Electron microscopy of donor endothelium

Peripheral donor endothelium was analysed in every case of cryopreservation or storage in M H medium. For ultrastructural analyses a piece of the peripheral corneal rim was fixed in glutaraldehyde with 0.1 M phosphate buffer pH 7.3. The tissue was post fixed with osmium tetroxide in phosphate buffer. The specimens were dehydrated in alcohol and carbon dioxide embedded in Epon resin and sectioned with a Reichert ultramicrotome. The sections were post stained with lead citrate and uranyl acetate. Cell morphology and the integrity of the cell structure were analysed at the Department of Electron Microscopy, University of Leuven with the JEM 100 S electron microscope.

Preparation of donor endothelium for scanning electron microscopy

Specimens for scanning electron microscopy (SEM) were prepared from 5 cryopreserved and 3 fresh corneas stored in M H medium. Pieces of the peripheral corneal rim were fixed in glutaraldehyde with 0.1 M phosphate buffer pH 7.3, post fixed in phosphate-buffered osmium tetroxide, dehydrated in a graded series of water-ethanol and critical point dried in CO₂. The dried tissues were then fixed to specimen stubs with conductive silver paint, sputter coated with carbon and gold in a Balzer BA 3 evaporator. Specimens were examined in a JEOL JSM L3 scanning electron microscope at 20 kV.

Results

The mean cell loss in each preservation group is shown in Table I. The mean endothelial cell loss for the 16 corneas was 49%. The mean cell loss for the 12 cryopreserved corneas was 50%. In the four other patients in whom the donor cornea had been stored in M H medium or transplanted fresh, the mean cell loss was 32%. The two fresh corneas had a mean cell loss of 42%. For the two corneas stored in M H medium the mean cell loss was 22%. For all preservation groups the mean post-operative period was 11.12 months. The mean donor age was highest (56 years) for the cryopreserved material.

Table II shows the different parameters for each individual case: donor age, recipient age, type and time of preservation, post-operative period, donor and graft endothelial cell density and the percentage cell loss in each case.

For the cryopreserved corneas from melanomatous eyes the cell loss during preservation and keratoplasty varied from 33% to 77% (Fig. 1A,B). No correlation existed between length of preservation and endothelial cell loss: in case 1, for example, with the longest cryopreservation time (3 years), cell loss was only 33%. The two highest cell losses were in cases 11 and 12. The latter patient had a vascularized lime burnt cornea, which is known to be one of the most difficult cases with a poor prognosis. The former, with keratoconus, had a complicated post-operative bacterial infection. In the two freshly transplanted corneas used as controls for the effects of cryopreservation and storage, cell losses were 40 and 44%. The transplanted endothelial

Table I
Endothelial cell densities (\pm SD) of donor corneas and grafts

Method of preservation	Number of donor eyes	Mean endothelial cell density of donor corneas (cells/mm ²)	Mean endothelial cell density of transplants (cells/mm ²)	Mean cell loss (cells/mm ²)	Cell loss (%)	Mean postop period (months)	Mean age of donors (years)	Mean age of recipients (years)
Cryopreservation	12	2539 \pm 308	1149 \pm 440	1384 \pm 386	55	11	55	34
Others	1	2861 \pm 217	1944 \pm 211	925 \pm 493	32	12	45	16
(M medium)	(7)	(2700 \pm 111)	(1113 \pm 88)	(538 \pm 53)	(27)	(12)	(45)	(38)
(fresh)	(5)	(3037 \pm 35)	(177 \pm 106)	(1262 \pm 71)	(42)	(12)	(41)	(6)
Total	13	2517 \pm 318	1317 \pm 520	1261 \pm 427	49	11	53	37

Table II

Sur No	Method of preser- vation	Length of preser- vation	Donor age (years)	Donor mean endo- thelial cell count (cells/mm ²)	Mean graft endo- thelial cell count (cells/mm ²)	Cell loss %	Lost opera- tive period (months)	Recipient age (years)	Recipient Diagnosis	Vase
1	crysto	3 y	70	9908	1125	93	2	99	Conus	-
2	-	7 mo	97	3000	2100	34	15	24	Conus	-
3	-	4 mo	97	9637	1710	94	2	17	Conus	-
4	-	7 mo	59	2400	1925	19	6	40	Conus	-
5	-	8 mo	70	1463	1900	1	14	97	Conus	-
6	-	6 mo	69	1975	889	55	1	97	Conus	-
7	-	8 d	21	9512	1100	50	96	64	Maculæ c	+
8	-	15 mo	7	9418	1017	39	9	35	Conus	-
9	-	1 mo	47	280	1150	60	9	11	Parench k	+
10	-	3 y	66	9716	818	69	2	99	Conus	-
11	-	1 d	37	9412	600	75	19	16	Conus	-
12	-	6 d	64	910	600	77	9	94	Corrosion	+
13	M K	3 d	31	9600	9050	21	10	91	Conus	-
14	M K	1 d	69	9800	917	22	19	19	Parench k	+
15	Fresh	-	37	9099	1850	40	12	18	Maculæ c	-
16	Fresh	-	31	9010	1700	11	11	60	Phlyct k	+

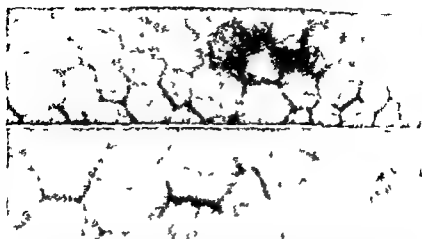



Fig 1 A B

A Central corneal endothelium of the donor before enucleation of the melanoma
The same endothelium photographed from the graft transplanted after storage in
1,5 months The cell densities are 2458/mm² in A and 1017/mm² in B (x 450) 

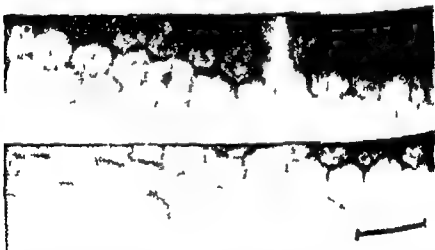


Fig 2 A B

A Central corneal endothelium on specular microscop of a melanomatous donor cornea
The same endothelium photographed from the graft transplanted after storage for 3 days
in M H medium Follow up period 10 months The cell densities are 2050/mm² in A and
2050/mm² in B (x 450) Bar = 50µ

tain the highest densities in the two corneas stored in M K medium with preservation times of 1 and 3 days. In these cases cell loss was only 21 and 22% (Fig

3 after staining

Fig. 3 of the peripheral endothelium of corneas stored in M K medium revealed normal hexagonal cell pattern. Different fields of view showed that all the cells were evenly stained and that in each cell the intensity of the dye was uniform. Thus



Fig 3 A B C

transmission electron micrograph of human corneal endothelium preserved in M K medium for 3 days. Membranes of cells and nucleus (N) appear undamaged but some vesicles (V) and perinuclear swelling (as arrow) are seen. The lateral cell borders are normally fused and closely apposed (arrows). Tight junction (TJ) is intact (x 6 000). B Higher magnification of the same endothelium showing closely apposed condensed cell borders (N) and tight junction (TJ) of adjacent cells (x 13 500). C Human corneal endothelium preserved in M K medium and stained after preservation for reduced nicotinamide adenine dinucleotide dehydrogenase. Uniform precipitation of the dye gives normal looking endothelial cells.

A



B

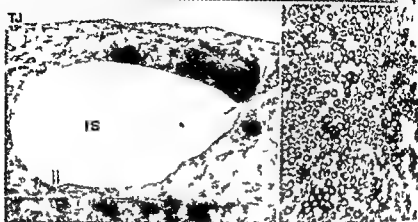


Fig. 4 A B C

A Transmission electron micrograph of cryopreserved human corneal endothelium. Intercellular spaces (IS) are highly enlarged and the cell membranes of adjacent cells are separated from each other (arrows). Also some mitochondrial swelling and vacuoles in the cytoplasm are to be seen. Cell membranes (arrows) and the nucleus (N) are visible. The Descemet's membrane (DM) is seen in the lower position ($\times 3500$). B Higher magnification view of the intercellular space (IS) seen in Fig. A. Cell membranes are attached to each other at the junction (TJ). Macula adhaerens (arrow) and near the Descemet's membrane (DM) ($\times 12000$). C Cryopreserved human corneal endothelium stained for reduced nicotinamide adenine dinucleotide diaphorase. Groups of unstained (dark) cells are present among normally stained cells.

in the two corneas stored in M.K. medium the viability of the endothelium evaluated by nitro blue tetrazolium staining was 100% (Fig. 3C). There was no difference in viability between endothelia stored for 1 and 3 days.

Similar staining was done on the peripheral rim of the cryopreserved cornea ($N = 12$) after thawing and punching of the central graft. In these corneas cryopreserved endothelial cells showed morphological variations. Furthermore, the intensity of the stain inside the cell cytoplasm varied and some cells had vacuoles. Those corneas ($N = 3$) in which mechanical trauma was suspected were eliminated from the analysis. The percentage of non-stained cells in the cryopreserved corneas was sometimes as high as 15% when counts were made on an average of 5 different fields of view (Fig. 4C) corresponding an area of 0.2 mm^2 in surface.

*Results of electron microscopy**Scanning electron microscopy*

revealed an intact endothelium in the periphery of the cornea in both cases in which the corneas had been stored in M K medium. There was no pleomorphism and cell borders were well seen (Fig 5 A). The nuclei of the preserved cells were visible. No distinct difference could be seen between the two corneas stored in M K medium, one for 1 day and the other for 3 days. Only an occasional ruptured or lysed endothelial cell was found among the normal looking hexagonal cells. In both cases it was observed that the endothelial cells formed a continuous lining on Descemet's membrane and had maintained their ultrastructural integrity.

In 5 cryopreserved corneal endothelia studied, most samples showed clearly defined cell borders. Moreover, the polygonal cells had prominent nuclei. There were large areas where the endothelial cells were normal looking and apparently intact, but also patches where they were disrupted. Where disrupted and lysed cells were seen, they were commonly grouped in rows or clusters of 5-10 cells. In these disrupted cells the nucleus and cellular organelles were exposed. No correlation was seen between the number of disrupted cells seen in SEM and the time of preservation (Fig 5 B).

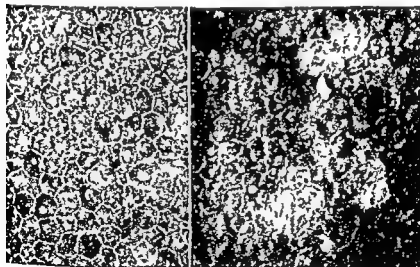


Fig 5 A B

Scanning electron micrograph of human endothelium preserved in M K medium for 12 months. A Normal mosaic like polygonal endothelial cells with intact cell membranes and nuclei. B Cryopreserved (8 months) human corneal endothelium. In this area some groups of cells are disrupted and swollen among normal looking hexagonal cells ($\times 350$).

Transmission electron microscopy

The peripheral endothelium of the corneas that were stored in M H showed relatively normal cells with dense nuclei and cytoplasm. The cells were normal in thickness. The plasma membranes were intact and the nuclei were mostly small and normal looking. The intercellular membranes were intact with tight junctions and maculae adherentes. Only a few granules were seen in the endothelial cells stored in M H medium. These granules were probably mostly mitochondria which had lost their inner structures. Small vacuoles were also seen between the endothelial cells and Descemet's membrane. The lateral membranes of the intercellular borders were closely apposed (Fig 3 A B).

In those corneas which had been cryopreserved for various times (frozen and thawed) a great variety of changes were seen in the endothelium. In most cases the nuclei and the endoplasmic reticulum showed no changes. On the other hand in almost all intercellular spaces the lateral cell membranes separated so that there were empty spaces of various sizes between the cells (Fig 3 A B). The cell membranes were intact and the cells were often attached to each other in one (tight junctions) or many places (maculae adherentes). Large vacuoles (enlarged mitochondria) were also found inside the cytoplasm. Often the cell membrane was partly detached from Descemet's membrane and the spaces of various sizes were found between the membrane and the endothelial cells. Small spaces were also found between the lateral borders of the cells.

Discussion

According to numerous reports the cell density of the corneal endothelium decreases after several kinds of intra-ocular operations but endothelial cell loss even when heavy is not immediately disastrous for the cornea remains viable with a very small number of endothelial cells (Bourne & Kaufman 1973; Lachman et al 1976a; Bron & Brown 1974).

In this study I measured endothelial cell densities before and after keratoplasty and so evaluated the cell loss during excision, preservation and transplantation of the graft. Counts were available for the exact numbers of endothelial cells in the donor or donor's fellow eye corneas before keratoplasty and in the transplants about one year later. The cadaver time with which cell loss is known to be minimal for all transplants. The role of histocompatibility was minimal for the patients had 0/1 mismatches and only 5 keratoconus patients had two mismatches. So the most important factors responsible for cell loss were the type of preservation of the corneas, the surgical manipulation and perhaps the age of the donor.

During cataract extraction the corneal endothelium suffers damage and the cornea is bent (Vorn 1971). In my study no endothelial cell damage was detected with vital staining in corneas stored in M H medium so that trephining the corneal button with a rotor trephine is harmless to the endothelium when done skilfully. Hence the cell loss seen in cryopreserved corneas is preservation presumably depends chiefly on the injury induced by dehydration, freezing and thawing.

Van Horn et al (1969, 1972, 1973) made studies on the effect of cryopreservation on the ultrastructure of the endothelial cells. They concluded that the cryopreservation method of Kaufman & Capella the viability of endothelial cells is maintained providing that the corneas are frozen within a few hours of the death of the donor. However they found large intercellular spaces between the endothelial cells which they attributed to the high osmolality of the medium used for corneal cryopreservation, a solution containing dimethylsulphoxide, glucose and albumin. Their study (Van Horn et al 1969) showed that incubation of corneas in this medium led to distended intercellular spaces. After freezing and thawing the spaces were no longer visible and the intercellular spaces apposed.

In the present study I found that the intercellular spaces were still attached to each other only minor morphological changes were seen. Grafts long after keratoplasty the dehydrating ion pump is still half the endothelial cells appear that most of the changes are reversible and that sooner or later normal function is restored.

SEM showed some cell loss in lines or clusters of 5-10 cells the result of mechanical damage. Disrupted cells the endothelium appeared less continuous. This seems reasonable looking in SEM no longer continuous.

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VITAL STAINING OF EXTERNAL EYE OF RABBIT by Fluorescein Rose bengal Tetrazolium and Alcian Blue

BY

M. S. NORN

Fifty normal albino rabbits (Sic. CPH) had 1% fluorescein and 1% rose bengal instilled into the right eye and 1% tetrazolium and 0.25% alcian blue into the left eye.

The cornea was stained by fluorescein in no more than 9% by rule but frequently (48%) and intensely often over the area coverable by the nictitating membrane and by tetrazolium correspondingly in 28%.

Rose bengal stained the nictitating membrane and often foliolar along its border.

Marx line was seen to be regular festoon shaped. Unlike the human line that of the rabbit does not continue through the punctum lacrimale but is 3 mm behind the lid margin at the nictitating membrane. The passage of dye takes a few minutes.

The alcian blue stained mucous thread resembled the human one. Percentage values and mean staining grades have been stated for the individual regions.

Key words: vital staining fluorescein rose bengal tetrazolium alcian blue lacrimal drainage system - nictitating membrane - Marx line - rabbit.

It is a well known fact that we should be wary of drawing conclusions from results of rabbit experiments to the clinical conditions in man. Not least in ophthalmic experiments: experimental keratitis and trauma. The external eye of rabbit differs in several important respects from that of man. The rabbit has an almost ring shaped lacrimal gland. It has only one punctum lacrimale 3 mm behind the lid margin at the root of the semilunar fold. The lacrimal

oped into a nictitating membrane which may cover at least half of the cornea rabbit very rarely blinks (Prince 1964)

may therefore expect to find vital staining properties in rabbit eyes differing from those in human eyes

object of the present investigation has been to compare the vital stainability rabbit eye with that of the human and in this connection to set up a normal value of rabbits with regard to the most important dyes serving for vital staining external eye

Material

albino rabbits (*Oryctolagus cuniculus*) had both eyes vital stained. The animals represent a cross breed between Danish country breed and ram rabbit from the closed colony since 1950 international term Ssc CPH kept in single cages State Serum Institute Copenhagen weights ranging from 2 to 3½ kg

Methods

right eye was vital stained by a mixture of 1% fluorescein and 1% rose bengal then rinsed with a balanced salt solution (Alcon) (N = 50). The left eye was stained by a mixture of 1% tetrazolum and 0.25% alcian blue (N = 50) without frequent rinsing (Norm 1974)

The rabbit calmed down and fixed by the keeper was examined without anesthesia in Kowa's hand held slit lamp (magnification × 20). A cobalt filter was in front of the light source for fluorescein and white light for the other dyes. Staining was graded on the basis of the number of stained dots within the area concerned (cornea, bulbar conjunctiva etc. - vide legend to Table I) grades corresponding to <30 <100 <1000 <10 000 and >10 000 dots respectively

Results

Vital staining properties of the external eye of the rabbit differed from the human in the following respects

1. The cornea was stained more often (Table I) and with a higher mean grade (Fig. 1). Rose bengal and tetrazolum (cf. Norm 1970, 1972) especially the entire medial or medio inferior half of the cornea, where the staining presented an upward

Table I

Vital staining of normal rabbit eyes ($N=50$). The figures indicate the percentage of eyes stained within the region concerned. C: cornea, B: bulbar conjunctiva, P: nictitating membrane, CA: caruncle, T: inferior tarsus, M: Marx line on lower lid, TS: superior tarsus, MS: Marx line on upper lid, MT: mucosa of inferior fornix of conjunctiva.

	C	B	P	CA	T	M	TS	MS	MT
Fluorescein	2	0	0	8	0	0	0	0	0
Rose bengal	48	2	92	84	2	100	19	90	1
Tetrazolium	28	0	6	8	8	16	19	19	1
Alcian blue	0	0	8	6	0	0	0	0	0

and temporally convex curve sharply demarcated from the remaining normal cornea.

The vital stained area was identical with the region coverable by the nictitating membrane. The delimitation seemed to correspond to the edge of the membrane.

The nictitating membrane was stained more often than the corresponding fold of the human eye by rose bengal. In 28% the more intense staining

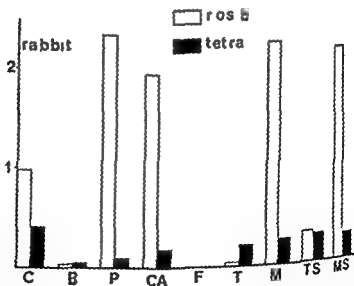


Fig 1

Vital staining of normal rabbit eyes by rose bengal and tetrazolium. Ordinate: percentage of eyes stained. Abscissa: sites (for abbreviations see Table I).

- ntrated particularly in the follicles located in a single row along the border of membrane. In another 28% the staining was seen as a band along the corneal border with no relation to follicles. In 28% as diffuse punctate staining, remaining cases as one or more horizontal bands.
2. *Marx line* along the lid extended as forward bulging festoons of rose bengal stained the outlets of the Meibomian glands often in several rows backwards. The arrangement was far more regular than in human eye where the outlets often much less regularly located.
3. *Marx line* was seen not to continue through the punctum lacrimale into the lacrimal sac as it often does in man. The rabbit's punctum lacrimale is situated 3 mm from the lid margin. This region was not stained at all and Marx line remained unchanged in the neighbourhood of the punctum lacrimale.
4. Rose bengal and alcian blue dye passed out of the nose few minutes after the instillation. In humans with a normal function this does not occur until 15-30 min.
5. The cornea was stained rarely but more often by tetrazolium compared with normal human eyes.
6. The mucous thread was stained by alcian blue and tetrazolium like humans. In only a few cases did the red colour predominate as a sign of bacterial infection (cf. Norm 4). In one of these the keeper had diagnosed inflammation of the eye prior to vital staining.
7. The fluorescein pattern was not detectable by rubbing nor by using Perkins ophthalmometer with positive pressure. Such has always been provokable in man (Norm 18). The difference is due to absence of Bowman's membrane in the rabbit.

DISCUSSION

The present series of 50 rabbits may be regarded as a normal material: the rabbits were well-cared for in single cages with no signs of corneal lesions (only one case of fluorescein stainability) or infection (though two with mucous thread staining suggesting infection).

Rabbit corneae were frequently stained by rose bengal presumably owing to absence of the nictitating membrane which in itself presents peculiar staining patterns. The festoon shaped Marx line and the lacrimal drainage system with its rapid outflow likewise differ essentially from the conditions in man. To these may be added a series of minor differences indicating that experience gained from human eye is not directly transferable to rabbit eye material nor vice versa.

Acknowledgments

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A MEDICATION MONITOR AND FLUORESCEIN TECHNIQUE DESIGNED TO STUDY MEDICATION BEHAVIOUR

BY

■ E NORELL¹ P A GRANSTRÖM³ and R WASSEN³

Methods were developed to study medication behaviour in order to obtain accurate and detailed information on the patterns of drug taking in medication with eye drops. A medication monitor was designed which recorded the date and hour each time the medication bottle was opened. A fluorescein technique was designed to study the ability of patients to administer the eye drops into the conjunctival sac. Some problems in the measurement of medication behaviour are discussed. In a group of patients with open angle glaucoma for whom pilocarpine eye drops three times daily had been prescribed 18% of dose intervals had a duration of 12 h or more and 11% had a duration of 4 h or less. Fluorescein tests indicated that the patients were usually able to administer the eye drops correctly.

Keywords: medication — patient compliance — pilocarpine — glaucoma.

In recent years much attention has been paid to patient compliance in general and medication behaviour in particular. Several studies have shown that about 50% of patients on long term medication do not follow the prescribed regimen. Higher or lower percentages have been found e.g. in different patient populations and with different drug regimens (Sackett 1976).

Two major problems in studies of medication behaviour are related to measurement methods. Firstly the accuracy has been shown to be unacceptably low for some of the most commonly used methods including interview (Bergman & Gerner 1963, Gordis et al 1969, Roth & Caron 1978). Secondly most of the methods used offer only very rough measures of the patterns of drug taking such

as the average number of missed doses. However, the effect of drug taking is not only related to the number of doses taken but also to the spacing between doses.

Different objective methods have been used to study medication behaviour. Among these, a medication monitor technique may offer the most accurate information on the patterns of drug taking. A source of radioactive material and photographic film was used by Moulding to monitor self-medication with ophthalmic drugs (Moulding 1962; Moulding et al 1967) and oral contraceptives (Moulding 1971). A medication monitor for eye drops was described by Lee (1971). The use of a medication bottle was recorded by an electronic system provided that the patient replaced the medication bottle in a container each time it had been used. This monitor was only used to monitor medication behaviour of two patients (Lee et al 1974).

For some patients and drug regimens there may be difficulties in administering the drug correctly. This problem is often neglected in studies of medication



Fig 1
Medication monitor with a 2.5-ml bottle for eye drops

four. Nevertheless it may be worth considering for example in medication with eye drops among elderly people. As far as we know no studies have been published on the ability of patients to administer eye drops into the conjunctival sac. The purpose of the present study was to develop methods to measure medication behaviour in order to obtain accurate and detailed information of the patterns of taking in medication with eye drops.

Material and Methods

A medication monitor was designed which recorded the date and hour each time the medication bottle was opened. The monitor (Fig. 1) consists of a plastic box measuring $100 \times 50 \times 25$ mm with a holder for a 20 ml medication bottle. The holder is designed to protect the bottle and to facilitate replacement of the dropper cap. An elastic flap linked to a micro switch inside the box signals to the electronic part of the monitor whether the cap is on or off. A sliding lid in the front of the monitor can be removed for exchange of bottle and battery (Fig. 2). The lid is sealed when the monitor is used.

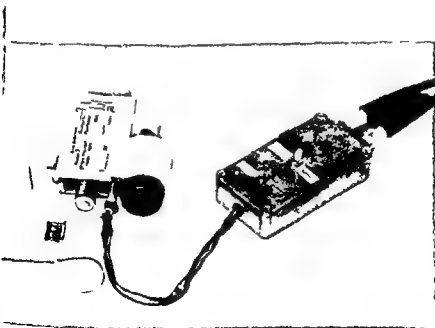


Fig. 2

Medication monitor with read-out device. Sliding lid and actuating plug removed.



Fig 5

Monitor record describing 20 days of tid medication. Each line represents a day. On each line representing the first hour after midnight and so forth. Each 'X' mark is preceded by a small vertical line indicating that the medication bottle was opened.

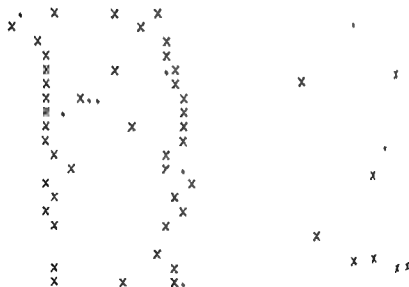


Fig 6

Monitor records of two patients for whom pilocarpine eye drops three times daily were prescribed. For interpretation see legend for Fig 5.

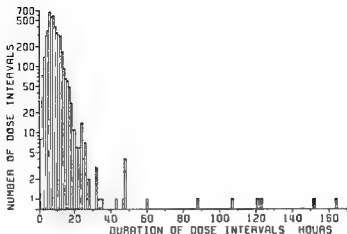


Fig 7

Frequency distribution of dose intervals in hours during a 20-day period for 82 patients for whom pilocarpine eye drops three times daily had been prescribed

Results

The display from the monitor by an ECG recorder is shown in Fig 4. The data from Fig 4 were fed to a computer and expressed on the first three lines of the printout together with the following 17 lines covering a 20-day period. Similar data for two other patients are shown in Fig 5. Monitor data on the duration of dose intervals from all 82 patients are given in Fig 7. The intervals varied from 1 to 164 h with a mean of 8.7 h and a maximum frequency at 6 h. Of the dose intervals 840 (5.9%) had a duration of 12 h or more and 509 (11%) had a duration of 4 h or less. All rescan tests were all positive except in one patient who had two negative tests.

Discussion

In the interpretation of medication monitor data some problems should be considered. For example, there could be variations in medication behaviour related to the design of the medication bottle and the duration of intervals between clinic visits.

The capacity of the monitor is limited to about three weeks and therefore the intervals between clinic visits will be shorter than usual for most patients.

The possibility of removing the drug from the bottle for consumption on a later occasion should be considered in medication with pills. However, in studying

medication with eye drops this is not likely to be an important problem. Variations in the number of doses taken on one occasion seem to be of less importance in medication with eye drops (Duke Elder 1967).

The fluorescein tests showed that with one exception our patients could not administer the eye drops correctly. This finding indicates that the monitor can supply information on dose intervals. Provided that the bottle was not used for some purpose other than trying to take the drug and that the patient did not have access to other sources of the drug.

During the time we have used the medication monitor there were no technical problems. In a few cases, however, a monitor record was obtained after a second 3 week period owing to defective monitor batteries during the test period. For practical reasons the medication monitor is useful for research purposes on a relatively small scale. Furthermore, the ethical and medication monitoring deserve further attention.

We believe that the medication monitor and fluorescein techniques can provide valuable information on medication behaviour since they offer data on the time of drug taking by date and hour. Such information could be an aid towards understanding and improving medication behaviour.

Acknowledgment

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REFRACTION NEARWORK AND EDUCATION A Population Study in Newfoundland

BY

AVRUM RICHLER and JOHN C BEAR

There is little information directly relating ocular refraction and near habits in representative human populations. Ocular refraction (diopters), nearwork (hours per day) and education (years) were therefore measured in 957 persons comprising 80% of the population aged 5 years and older of 3 communities in western Newfoundland. Refraction was moderately consistently and significantly correlated with nearwork from ages 5 to 13 and remained so after adjustments for the association of refraction and nearwork levels with age, sex and education. Multiple regression coefficients relating refraction to nearwork decreased from -0.13 D/h at ages 5-14 years to -0.02 D/h at ages 60 years and up. The magnitude of this association and its consistency and persistence over a wide age range suggest that large amounts of nearwork in childhood may contribute to the prevalence of clinical myopia.

Key words: refraction - nearwork - education - population study - epidemiology

Ocular refraction in man is a continuously distributed attribute usually partitioned arbitrarily into myopia, emmetropia and hyperopia. Its distribution is roughly normal but leptokurtotic with a mean and mode in the low hyperopia and a deficiency in the low myopia range (Goldschmidt 1971). The distribution is markedly leptokurtotic still exhibiting a mean and mode in hyperopia but also a pronounced skew to myopic values (Beuch 1972).

Myopia of clinical degree usually becomes manifest in late childhood or

in degree for some years thereafter (Duke Elder 1970). In its higher degrees it is of particular importance because it can often progress to blindness. High myopia is usually pathological rather than an extension of normal variation in refraction (Francois 1961, Duke Elder 1970). Lesser degrees of myopia are also of considerable social and economic importance. Over half the population of the world over the age of 15 years wear corrective lenses; of these about half wear lenses correcting for myopia. Nearly 90% of corrections worn by those between the ages 15 and 27 years are for myopia (National Center for Health Statistics 1978).

The complexity of the eye and of its refractive elements is such that its growth and development must be under elaborate genetic control. Emmetropia, which occurs more often than would be expected were refraction normally distributed, results from correlated growth of cornea and lens adjusting for elongation of the axial axis (Sorsby et al. 1961). Resemblances between relatives suggest a substantial genetic component in population variation in ocular refraction (Sorsby et al. 1962).

On the other hand, the morphological variations underlying substantial refractive differences is very small. An uncompensated excess of 1 mm, i.e. 4% in the length of the optical axis, which averages 24 mm in adulthood, implies a myopia of -3 D. Only about 10% of the U.S. population are as shortsighted or more so (National Center for Health Statistics 1978). Subtle environmental disturbance of ocular development might therefore markedly influence refraction.

There is experimental evidence that in accommodation, the process by which the eye adjusts to near viewing, the tension on the choroid is increased (van Alphen 1961) and it has been postulated that a resulting increase in intraocular pressure causes the optical axis to elongate and the eye to become myopic (Young 1975). Thus it is reasonable to postulate that variation in nearwork levels among the young could, through the influence of accommodative effort on axial length, contribute to population variation in ocular refraction.

There is relatively little information relating ocular refraction and nearwork in representative unselected human populations. In Danish male conscripts myopia is clearly associated with occupational and educational background, more educated groups having a higher frequency of myopia, and this association has persisted over the last 80 years (Goldschmidt 1968). Myopia is more common in Finnish children in academic as opposed to other school streams (Goldschmidt 1968) and in British children of non manual as opposed to manual workers (Cockham et al. 1977). Genetic heterogeneity of these populations along social class lines may be at least suspected of confounding these observations (Goldschmidt 1968).

In view of the lack of direct observations in this area and because of its potential importance, an investigation was undertaken relating the ocular refraction, educa-

tion and nearwork habits of inhabitants of 3 rural Newfoundland communities. Familial resemblances in refraction and in nearwork habits were also noted. Results of these studies are reported elsewhere (Bear & Richler 1971, in publication in J. biosoc. Sci.).

Subjects and Methods

The population studied resides in 3 communities situated within 91 km of each other on the west coast of Newfoundland and is historically and geographically stable with little immigration. Data were collected in 1974.

Ocular refraction was determined in the course of a standard eye examination of each subject (Borish 1970). All refractions were performed by the same observer (AR). A fogging lens was used to relax accommodation. Refraction was evaluated with the retinoscope and refined subjectively both monocularly and binocularly as the minimum concave or maximum convex lens in dioptres required to give a distance acuity of 20/20. For purposes of the present study, astigmatism requiring cylindrical correction was converted to spherical power in the 'plus' meridian and because right and left eye refractions are highly correlated in this series ($P < 10^{-5}$) only right eye refractions were analyzed.

Nine hundred and seventy-one (971) persons aged 5 years or over were refracted, about 80% of the total population above that age. All subjects were refracted by the same observer (AR). Subjects were not selected on visual criteria. Eleven persons with myopia greater than -6D were excluded from further analysis because of the high probability that their myopia was pathological. Three persons with right eye amblyopia were also excluded. The population and its distribution of refraction are described elsewhere (Richler & Bear 1971).

Nearwork was measured as h/day as reported by the subject spent in activities such as reading, sewing or knitting requiring focussing of the eyes at a distance of 20 inches or less. This measure is clearly approximate and depends upon accuracy of recall and the habitual attentiveness of subjects in nearwork tasks. The 971 persons to whom this analysis relates comprise 388 young school leavers, 106 housewives, 106 fishermen, 44 men occupied in farming, forestry or fishery, 44 men working in construction trades or as truck drivers, 23 sales clerks, 23 as bookkeepers, health personnel, draftsmen or mechanics, 14 teachers, 14 plant workers and 26 persons giving no occupation. It can be appreciated that a small proportion of the population were engaged in occupations requiring moderate amounts of close work or reading.

Education was measured in years as last completed grade. Formal education effectively became compulsory only after the confederation of Newfoundland

In 1949 a change affecting persons aged 30 years and under at the time of investigation. Many subjects above middle age had little or no formal education. Those who have returned to school for upgrading in primary education grades so completed are included in the education measure.

Age was calculated as (year of investigation) - (year of birth). Sex coded for subjects as 1 = male 2 = female.

Table 1 presents means and standard deviations of refraction, nearwork and education by five year age intervals. Refraction means trend to more negative (myopic) values between the age of 5 and 15 years, trend to more positive (hyperopic) values from age 20 to age 59, and take an erratic course at higher ages. The pattern of this pattern and of the introduction of compulsory education in 1949. The population was divided in 5 groups by age: 0-14, 15-29, 30-44, 45-59 and 60 and up. The relationship of refraction to the other variables and to sex, was examined in each of these age intervals.

Results

Table II presents the simple correlation coefficients relating refraction to age, sex, education and nearwork in each of the 5 age intervals. (It may be noted that since the effects of negative power correct for myopia, negative correlations with refraction imply a positive association with nearsightedness.)

The numerous significant correlations are for the most part explicable. The tendency to myopia in the first age interval is generally observed, as is a gradual tendency toward hyperopia after that period (Duke-Elder 1970, National Center for Health Statistics 1978). The positive associations of education and nearwork with age in the youngest group result from school attendance among persons aged 0 to 14; the association of education with age is negative because only rudimentary formal education was available to the older subjects. The association of education and nearwork is hardly surprising. Associations of nearwork and sex probably indicate greater interest in school and home activities among young girls than among boys, and time spent in knitting and sewing by older women. The association of refraction with sex in the 15-29 year group indicates a generally observed sex difference in age changes in refraction, the shift to negative refractions with age occurring earlier in females than males, and being somewhat greater (Young et al. 1964, Sorsby et al. 1961, Goldschmidt 1968).

Most striking is the consistent association of refraction with nearwork until age 44. This association is maintained despite a reversal in the relationship of nearwork

Table I
Age distribution of refraction, nearwork and education

Age interval	n	Refraction		Nearwork		Education
		mean	SD	mean	SD	mean
5-9	195	-0.620	1.026	0.646	0.814	5.5
10-14	145	-0.172	1.251	1.435	1.010	6.5
15-19	108	-0.924	1.472	1.796	1.188	10.4
20-24	76	-0.812	1.619	1.161	1.478	11.4
25-29	85	-0.296	1.722	1.471	1.111	11.56
30-34	53	-0.094	0.935	1.434	1.394	9.5
35-39	49	0.082	0.967	1.704	1.479	9.75
40-44	55	0.236	1.409	1.200	1.850	9.5
45-49	52	0.533	1.477	1.113	1.309	6.5
50-54	48	0.765	0.894	0.771	1.219	5.25
55-59	25	0.747	0.706	0.840	0.911	4.4
60-64	28	1.414	1.595	0.393	0.561	4.4
65-69	18	1.442	1.445	1.056	1.259	4.4
70+	20	0.758	1.608	0.850	0.875	4.4

to age in the teenage years and despite the more gradual but even greater increase in the relationship of education to age.

In the oldest group senile lens changes affect refraction in some cases and the smaller number of subjects may account for the general weakening of associations between variables after age 60.

Partial correlation coefficients (Table III) indicate that the confounding of age and sex inflates somewhat the simple correlation of nearwork and refraction in the 5-14 year interval but not at higher ages and suggest the association of education with refraction can account for some of the association of nearwork and refraction. The association of refraction with nearwork remains significant after age 60 after statistical adjustment of both variables for their association with age and education.

Multiple regression equations (Table IV) were calculated in each age interval to determine the proportions of population variance in refraction that can be independently attributed to differences in each of the other variables. The proportion of refraction variance was not accounted for by this analysis at any age. For persons aged under 60 years education and nearwork taken together account for substantial proportions of refraction variance and very substantial proportions of refraction

ince. Because education and nearwork are correlated, the proportions of unexplained variance allotted to each depend on the order in which they are entered in the regression equations. When entered before education, nearwork accounts for more variation than education in each age group (data not shown).

Table II
Correlations among refraction and other variables

	Age	Sex	Education	Nearwork
<i>15-14 (n=340)</i>				
Refraction	-0.374	-0.081	-0.399	-0.42 *
Age		0.040	0.962* *	0.409 *
Sex			0.074	0.276 *
Education				0.452 **
<i>15-29 (n=269)</i>				
Refraction	0.176	-0.215	-0.917	-0.33 ***
Age		-0.049	-0.036	-0.114*
Sex			-0.059	0.006
Education				0.913***
<i>30-44 (n=157)</i>				
Refraction	0.109	0.007	-0.384	-0.489* *
Age		-0.041	-0.353 **	-0.121
Sex			-0.049	0.006
Education				0.461
<i>45-59 (n=125)</i>				
Refraction	0.178	0.015	-0.187	-0.758 *
Age		-0.209	-0.299* *	-0.097
Sex			0.067	0.162
Education				0.534 *
<i>60+ (n=66)</i>				
Refraction	-0.238	0.166	0.007	-0.098
Age		0.140	0.170	0.63*
Sex			0.033	0.221
Education				0.496 *

$P < 0.05$ $P < 0.01$ $P < 0.001$

Table III
Partial correlations of refraction and nearwork

Age interval	Simple r	Controlling for age and sex	Controlling for age, sex and education
0-14	-0.472	-0.371**	-0.351
15-29	-0.352	-0.310*	-0.30
30-44	-0.189	-0.192**	-0.349*
45-59	-0.274	-0.259**	-0.199*
60+	-0.098	-0.082	-0.111

* $P < 0.05$ ** $P < 0.01$

Discussion

Present findings clearly indicate in the population studied an association between refraction and an admittedly approximate nearwork measurement. The question however whether this association indicates that nearwork actually *causes* refractive error is not clear. For instance it has been plausibly argued by Goldschmidt (1977) that the association of myopia with education and occupation in the relatively homogeneous structure of Europe is the result of prolonged genetic selection and stratification. On the other hand the prevalence of myopia in Eskimo and other populations seems to have increased dramatically with the introduction of education (Young et al. 1969; Woodruff & Samek 1977) at a rate far in excess of that sustainable by genetic mechanisms.

What social and economic differences exist in the population have been relatively minor as indicated by the distribution of occupations and the settled nature of the population. Lack of recent immigration and pre-genetic analyses (Marshall 1976) suggest it to be relatively homogeneous genetically. The observed associations of refraction with nearwork and education are therefore not easily explained in terms of underlying social or genetic variation.

The persistence and relative stability of the association of refraction and nearwork in each age interval and the strikingly parallel age trends of each (Fig. 1) despite reversals in the relationship of each with age suggest that the association is not artifactual. It cannot be argued that increasing levels of education in this population have increased the average person's nearwork in a manner which happens by chance to parallel age changes in refraction which can be seen in any case because as shown in Table III the association remains

Table IV
Multiple regression of refraction on other variables

Age interval	Independent variables	Multiple correlation	Variance described (r^2 change)	Regression coefficient	Standard error
<14	age	0.3739	0.1393	0.0079	0.0754
	sex	0.3797	0.0044	0.0499	0.1154
	education	0.4035	0.0187	-0.1138	0.0806
	nearwork	0.5159	0.1033	-0.4339	0.0631
	constant			1.0592	
15-19	age	0.1756	0.0308	0.0480	0.0904
	sex	0.2713	0.0498	-0.6698	0.1908
	education	0.3519	0.0503	-0.1257	0.0513
	nearwork	0.4260	0.0576	-0.3007	0.0698
	constant			1.1918	
20-44	age	0.1094	0.0120	-0.0097	0.0188
	sex	0.1100	0.0001	-0.0029	0.1588
	education	0.3851	0.1362	-0.0801	0.0324
	nearwork	0.5908	0.1229	-0.9868	0.0567
	constant			1.9669	
45-59	age	0.1280	0.0164	0.0316	0.0936
	sex	0.1348	0.0018	0.1828	0.2067
	education	0.2061	0.0243	-0.0094	0.0378
	nearwork	0.2903	0.0418	-0.9294	0.0980
	constant			-0.9424	
60+	age	0.2384	0.0568	-0.0536	0.0274
	sex	0.3123	0.0407	0.7367	0.4067
	education	0.3196	0.0003	0.0464	0.0137
	nearwork	0.3307	0.0110	-0.2948	0.2588
	constant			3.6548	

adjustment for the effects of age and education on refraction and nearwork level since it is highly improbable that in the population studied persons of all ages are carefully adjusting their generally low nearwork levels to their refractions these findings strongly suggest that nearwork does indeed influence refraction in a myopic direction. The exclusion of 11 subjects by virtue of their unusually high myopia does not affect the observed association of myopia with nearwork these persons had on average more education 10.18 years and did rather more nearwork 2.82 h daily than the rest of the subjects.

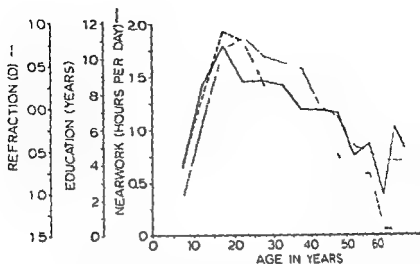


Fig 1

Course of 5 year age group means of refraction, nearwork, and education

It is not possible in cross sectional data to approach the question of whether nearwork levels decrease refraction in all young persons or only some. Longitudinal studies indicate that most children show increases in axial length with age the rate of elongation being variable. Such elongation uncorrelated with correlated cornea and lens growth is the principle cause of myopia. Compensatory growth of cornea and lens does however usually occur again to varying degrees and emmetropia is usually fairly well maintained. Compensation is less complete when the rate of axial elongation is high (Sorsby et al 1961). A variation in growth of the ocular components is superimposed on the refractive error which has some influence on the refraction measured in later childhood (Sorsby et al 1961, Hirsch 1964). The large excesses of clinical myopia observed among Eskimo and Amerind students by comparison with their peers suggest that levels of nearwork usual to North American education may be a cause of the refractions of most young persons.

The regression values relating refraction to education and nearwork in this population (Table IV) should be cautiously interpreted. In the individual refraction normally changes only gradually after late adolescence. The regression coefficients relating refraction with nearwork in each age group (Table IV) cannot be taken to imply that for members of this population a given amount of nearwork each day will on average decrease refraction by the same amount regardless of the age at which the nearwork is undertaken. The

Older and younger nearwork associations with refraction are more likely to indicate personal nearwork levels are indeed habitual, established in childhood and adolescence and that their effect on refraction is permanent. Pending confirmation of the findings of the present investigation in studies elsewhere the regression coefficients observed should not be extended to other nearwork levels in other populations. The magnitude of these regressions, -0.3 D per h of nearwork, implies higher levels of nearwork in childhood and adolescence may have clinically important effects on refraction, particularly in cases of moderately high myopia. In particular, longitudinal and family studies might clarify the generality of the nearwork-refraction association.

Acknowledgments

We acknowledge with gratitude the cooperation of the subjects of this report, the many assistants of the staff of the West Coast Health Survey (W. H. Marshall, Head), computer assistance from G. Burke, graphics work by Medical Audiovisual Services (MLN), clerical assistance by J. Dawe and J. Smith, and a critical reading of the manuscript by F. J. Cole.

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JUDICIA DE NOVIS LIBRIS

Die Ophthalmologische Gesellschaft Bericht über die 73. Zusammenkunft in Heidelberg 1977 Kunststoffimplantate in der Ophthalmologie Editor: W. Jaeger J. F. Bergmann Verlag München 1978 717 pages.

than 200 papers from the German ophthalmological society meeting in Heidelberg are presented in this book. Of the numerous papers 73 deal with the specific topic for meeting. Some papers discuss the pure technical aspects of polymers and plastics, others issue reaction to these materials from a clinical point of view. Great interest is also paid to ocular lenses.

Each discussion finishes by dealing with some aspects not mentioned in the papers. It is pointing that one of the greater complications with intraocular lenses, namely endo-ocular dystrophy, is only mentioned in one sentence.

The useful application of acrylic adhesives in detachment surgery and in combination contact lenses is beautifully presented in several papers. The results in detachment surgery using various implants are presented in 12 papers.

The main topic for the conference is completed with a discussion on iris-clip-lenses and capsular lenses in 20 pages with the very experienced pioneers in this field.

It is not possible in a brief review to mention the free papers. It is sufficient to emphasize almost any field in ophthalmology is presented.

This book with the many free papers can be recommended to any ophthalmologist.

T. ben Soensen

Visual Handicap in Children, Vernon Smith & John Keen eds. Clinics in Developmental Medicine No. 73 Spastics International Medical Publications with W. Heinemann 1979 172 pages Price £K Pds 8.00

The Spastics Society in 1976 sponsored a meeting on the visually handicapped children. The communications of the meeting have been published in this volume. The contents are as relevant today as they were in 1976 and contain the most up-to-date statistics on visual impairment in childhood.

The last report by the late Dr. Mary Sheridan concerning assessment of vision in small children and infants is also included and is as clear and persuasive as anything she has presented.

In fact, most problems concerning blind and partially sighted children are discussed: such as causes of blindness, common complications, genetics of the most prevalent causes of visual impairment, development of normal vision, developmental psychology of children with deficient vision, and how this affects the teaching of the visually impaired.

The present trend of integrated education has allowed educationally subnormal children to enter the previously very competitive schools for the blind, and several authors discuss visual handicaps among mentally retarded children. It will astonish some that 70% of blind children are multihandicapped.

The volume ends with a short but intense and comprehensive chapter by a mother to two visually retarded and one unaffected child. This chapter, at least, should be read by everybody in charge of visually handicapped children or their families. In unsophisticated phrases Mrs. Matthews explains why parents must have a diagnosis, genetic counselling and educational advice as early as possible, and that is what the rest of the volume is all about.

Mette Waaburg

A. F. Deutman and J. R. M. Crisberg: Neurogenetics and Neurobiology
International Congress Nijmegen The Netherlands September 6-1977
by Publishers The Hague Boston London 1978 486 pages 200 figs
Price Dutch Guilders 160 - US dollars 84 -

At the 5th international congress of neurogenetics and neurobiology on 6-12 September 1977 the main topics were myasthenia gravis, optic atrophy and degenerations. Furthermore there were free lectures on neuroophthalmology.

The first section of the book deals with myasthenia gravis and includes articles by Huber. Ocular myasthenia: diagnosis and treatment. In the subsequent articles of the disease are discussed - such as histology and histochemistry, acetylcholine myasthenia gravis - the treatment and finally the important genetic aspects.

The next 100 pages deal with diseases of the optic nerve and chiasma, including articles by J. Francois and W. Jaeger on the hereditary optic atrophies. In this book the various authors have also stressed the importance of the examination of diseases of optic nerve and chiasma. Among other things can Lach & Herdén value of visual evoked cortical response in optic neuritis and tumours within the visual pathways.

In the next passage there are excellent articles about the hereditary retinal diseases.

The final passage comprises a number of articles with quite different neurological topics.

On the whole the articles of the book are well written and it must also be many of them have an extensive and adequate bibliography enabling the reader interested in these subjects to obtain considerable information. The book is of interest to neuroophthalmologists and to those concerned with genetics and with retinal diseases. The book should find a place in the library of both eye and neurological departments.

Sensory Ecology: Review and perspectives edited by M. A. M. Menum Proulx
London 1977 297 pages Price £5.00

This extensive book is vol. 418 in NATO Advanced Study Institute series and contains nearly all the lectures from a congress in Canada July 1977.

The subject is the organ of vision and the evolution of other organs illustrating various studies including comparative anatomy of electrophysiological and visual patterns and also from an ecological standpoint. One is overwhelmed by the sensory organs, the well-disposed experiment of techniques and the many.

Unicellular organisms with a markedly developed eye organelle are described and birds can register polarized and ultra violet light snakes can remember. There is a description of the parietal eye - secondary adaptation of the terrestrial an aqueous existence - echo sense - electrical sense - magnetic sense - hygroscopy. The sense of smell is strongly developed in salamanders (the marker). Lunar neurohormones and the song of the grasshopper are other fascinating topics.

The material is presented in 19 well-defined chapters with few but very good. Each chapter is concluded with a status and a suggestion as regards future research.

The book ends with an extensive 60 pages index.

The book can be recommended to anyone wishing to become acquainted with the advances in the research of these exciting and amusing topics.

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HUMAN SENILE CATARACT

Light and electron microscopic studies of the
morphology of the anterior lens structures
with special reference to anterior capsular/subcapsular opacity

BY

O A JENSEN and A M LAURSEN

Light and electron microscopic studies of human senile cataractous lenses with and without biomicroscopically detectable anterior capsular subcapsular opacity (ACSCO) revealed the main difference between the two types of cataract to be in the subepithelial cortex where ACSCO lenses showed disintegrating cortex fibres fibres of the deep cortical type and even in some cases collapsed fibres. These findings were considered to be associated with the decomposition and disappearance of the superficial cortex in ACSCO lenses. Numerous mitochondria in the epithelium of ACSCO lenses point towards a high oxidative metabolism which may facilitate active transport across the epithelium.

No difference in capsular surface morphology between cataractous lenses with and without ACSCO was found by scanning electron microscopy. Transmission electron microscopy showed in both categories of lenses granular inclusions in the capsules most pronounced in totally opaque lenses.

Large intercellular vacuoles were seen in the anterior part of the epithelium both light and electron microscopically in both categories. Consequently these ultrastructural changes do not seem to form part of the biomicroscopical picture of ACSCO. Based on our study we prefer the term ASCO (anterior subcapsular opacity) in place of ACSCO the latter term having been used by us previously.

Anterior subcapsular cataract human senile biomicroscopically opaque anterior subcapsular (ASCO) microscopy light and electron cortex fibres subepithelial - degeneration

Received January 10th 1980

A biomicroscopically visible opacity located on the anterior aspect of cortex and characterized by the occurrence of vacuoles was reported by von Vogt (1914). Seen in the slit lamp this opacity (so far called by us the capsular/subcapsular opacity) appears as numerous capsular or subcapsular vacuoles so that the anterior aspect of the lens may even get a foamy appearance (Bruun Laursen 1976). The occurrence of ACSCO was primarily – though not exclusively – in the most opaque lenses (Klauder & Laursen 1977; Bruun Laursen & Fledehus 1979) has never been found as an isolated phenomenon in senile lenses.

Senile cataractous lenses with ACSCO – in contrast to cataractous lenses without it – are associated with physical and biochemical abnormalities such as a low Ca^{2+} (Bruun Laursen & Fledehus 1979), low whole lens ribonucleotide P^{32} (Laursen 1976) and high Ca^{2+} and low Ca^{2+} in whole lens water (Klauder & Laursen 1977).

The aim of the present study was to investigate the histopathology of ACSCO and also to find an appropriate name for the phenomenon. This was carried out by means of a) light microscopy (LM), b) transmission electron microscopy (TEM) and c) scanning electron microscopy (SEM).

Material and Methods

The study comprised 32 lenses from 31 patients with uncomplicated senile cataract, aged from 62 to 87 years. Fourteen lenses had immature cataract with ACSCO, 10 lenses had immature cataract without ACSCO and six lenses were totally opaque without ACSCO. LM and TEM were performed on all lenses, whereas SEM was performed after followed by the two other procedures.

Biomicroscopical evaluation was performed according to Bruun Laursen (1976) with mydriasis 1–3 days before operation, slit lamp photos being taken. The lenses were extracted in retrobulbar anaesthesia. The cataract extractions were without α -chymotrypsin was not used. The frozen extraction area was marked with ink. The lenses were rinsed in 0.9% saline and immediately placed in fixative. The lens remained for 4–24 h. The following fixatives were used at 1% glutaraldehyde (3 lenses), 4% glutaraldehyde (9 lenses) and equal parts of 1% glutaraldehyde + 2% formaldehyde (8 lenses). All fixatives were buffered with 0.1 M sodium cacodylate, pH 7.2.

The part of the lens to which the cryo-extractor had been attached and where the lens was cut away. Small pieces of the anterior structures (about 1 mm³) were further fixed in freshly prepared fixative for 2 h, postfixed in 0.1% OsO₄ for 1 h, cacodylate buffer, dehydrated in graded acetone and embedded in Epon 812. Sections of 1 μ m were stained with toluidine blue, examined and photographed in a light microscope. Ultrathin sections were cut with diamond knife in a Reichert Ultratome L3 and stained with 1% uranyl acetate for 45 min and 1% lead citrate for 3 min.

fixed in a Zeiss EM 9S 2 electron microscope at 60 kV or a JEOL 100 C at 60-80 kV. The majority of negatives studied were magnified three times. Magnifications used for illustration are indicated in the legend.

For SEM, the fixed, postfixed and dehydrated whole lenses were critical point dried in a critical point drying apparatus E.3000, the acetone being replaced by liquid CO₂ and heated through the critical point at about 1600 psi and 40°C. The specimens were fixed on stubs with colloidal silver and coated with 50 nm Au/Pd in a Polaron E 5100 Series II sputter. Scanning was performed in a JEOL JSM 30 at 15 kV.

For subsequent TEM, the lens was removed from the SEM stubs with a razor blade. A small piece of 1 mm² of the anterior surface cut out and then placed in a propylene epon mixture for 4 h in vacuum and for 2 h in pure epon before polymerization at 60°C for 48 h. Further processing as above.

Results

No significant difference was found between lenses fixed by the various fixatives or by the various concentrations.

In the following, the most important similarities and differences are reported between lenses with (immature and totally opaque lenses) and without ACSCO (mature cataractous lenses).

capsule

Light microscopy showed no difference between the capsule of the various types.

In TEM examinations, granular inclusions as described by Seland (1974, 1978) were observed in both categories of lenses, particularly in totally opaque lenses (Fig. 1A, C). No quantification was made, but no pronounced difference between immature cataractous lenses with and without ACSCO as concerns capsular inclusions was observed.

SEM revealed no difference between the surfaces of lenses with and without ACSCO.

epithelium

In the light microscope, cataractous lenses with and without ACSCO had, as a rule, no pronounced epithelial vacuoles. They were located predominantly in the anterior two-thirds of the epithelium (Fig. 2A, B). Here and there areas without vacuoles were seen – even in totally opaque lenses. In a few cases of total lens opacity, areas of epithelial atrophy with total cell loss occurred centrally.

In TEM, the vacuoles were identical in the two categories of cataractous lenses. They were seen as numerous large intercellular and a few intracellular vacuoles. The intercellular vacuoles contained small processes of cytoplasm, possibly detached from the wall (Fig. 3A), and such material often merged with electron-dense coated



Fig 1 A B C

- A Crystalline inclusion in capsule and epithelium. d = dense, v = vacuole. Total opaque lens. Lab N 315-8 TEM 3101 30
- B Minor granular inclusions in capsule of total opaque lens. arrow

C

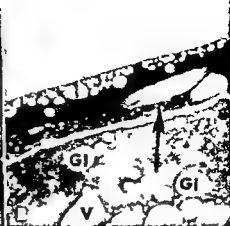


Fig 2 A B

ACSCO in immature cataract. Vacuoles in the anterior part of the epithelium (e). The epithelial cortex heavily changed with varying thickness of lens fibres and bladder cells of (W) and vacuoles (V). c = capsule. 1 μ m section. Toluidine blue. Lab No 44/8 ($\times 900$).

totally opaque lens. Epithelial (e) and cortical (V) vacuoles, globules (GI) and subepithelial amorphous substance (arrow). 1 μ m section. Toluidine blue. Lab No 608/8 ($\times 900$).

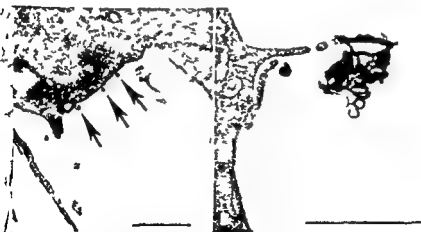


Fig 3 A B

small cytoplasmic bodies possibly being detached from wall of epithelial intercellular vacuole (arrows). Immature cataract with ACSCO.

Lab No 253/8 TEM 285A ($\times 14,400$).

Coiled string like masses possibly derived from cytoplasmic process. Wall of epithelial intercellular vacuole at left. Immature cataract with ACSCO.

Lab No 831/8 TEM 334A ($\times 28,500$).



Fig 4 1 B

A Intraepithelial vesicles (ve) in immature cataract with ACSCO m = mitochondria
Lab No 744/78 TEM 324 ($\times 28,500$)

B Intraepithelial vesicles (ve) and Golgi apparatus (G) beneath the nucleus (N). Cell
subepithelial fiber (arrow) Immature cataract with ACSCO Lab No 744/78 TEM
322-400

string like masses (Fig 3B). Nuclei were often compressed by vacuoles
irregular shapes in both categories (Fig 11). Small vesicles or intracellular
were observed in both categories particularly at the cortical end of the cell,
in connexion with the Golgi apparatus (Figs 4A, B, 8). Mitochondria with
cristae were seen in large quantities in all ACSCO lenses (Figs 4A, B, 6),
some lenses without ACSCO showed few mitochondria and in no case
mitochondria than lenses with ACSCO.

Dense bodies as described by Gorthy et al (1971) occurred in both
sometimes near or on the epithelio-cortical borders (Figs 1A, B, 8, 10).
Endocytotic vesicles were seen to open on the capsular as well as on the
aspect of the epithelial cell in both types (Fig 5D, E). Endoplasmic reticulum
to the nuclei as well as the Golgi apparatus also appeared identical in the
categories of lenses.



Fig 5 A B C D E

- subepithelial multivesicular body (mv) adjacent to flat cortical vesicle (ve) Totally opaque lens Lab No 688/78 TEM 323 ($\times 57\,000$)
- cortical multivesicular body (mv) on cortico-epithelial border (m = mitochondrion) Totally opaque lens Lab No 688/78 TEM 323 ($\times 57\,000$)
- cortical vesicle (ve) on cortico-epithelial border of totally opaque lens (m = mitochondria) Lab No 897/78 TEM 341 ($\times 44\,800$)
- exocytotic vesicles on the epithelio-capsular border (arrow) c = capsule Immature cataract without ACSCO Lab No 1128/77 TEM 239 ($\times 37\,000$)
- possible epithelial exocytotic vesicle on cortico-epithelial border (arrow) Immature cataract with ACSCO Lab No 744/78 TEM 394 ($\times 60\,000$) All scale bars $\bar{1}\,\mu\text{m}$

rtex

ht microscopically the subepithelial cortex was regular and uniform in lenses without ACSCO but heavily distorted in ACSCO lenses (Fig 2A B)

In the latter bladder cells of Wedl (Fig 2A) globules vacuoles and amorphous substance of varying density were usual (Fig 2A B)

By TEM the most anterior cortical fibre of all cataractous lenses was thinner and sometimes darker than the deeper cortical fibres (Figs 4B 6 7) The subepithelial fibres of ACSCO lenses were obviously of the deep (midzonal) type as described by Uwabara (1975) and Kobayashi & Suzuki (1975) As illustrated in Fig 6 they are

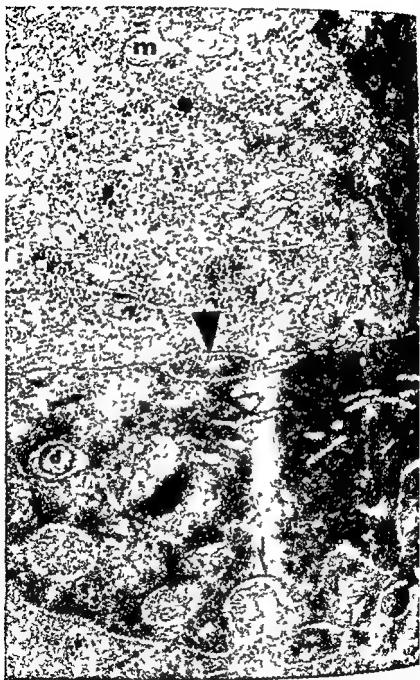


Fig 6

Interlocking processes (black arrows) circumscribed cytoplasmic material between epa and cortex (arrow heads) and intrafibril clefts (open arrows) d = cortical dense body epithelial glycogen m = epithelial mitochondrion N = nucleus Immature cataract
ACSCO Lab No 744/78 TEM 324 ($\times 57\,000$) Scale bar 0.1 μm



FR

the cataract without ACSCO showing regular lens fibres. Fibres immediately beneath the epithelium are dark. N = nucleus. Lab No 1199. TEM 999 ($\times 5000$)

characterized by many interlocking processes as well as by their irregular shape. In contrast, the subepithelial cortical fibres of lenses without ACSCO had few locking processes and a more regular shape (Fig 7). In the intercellular space between epithelial and cortical cells, small bodies, probably consisting of cytoplasm, sometimes observed in ACSCO lenses (Fig 6).

ACSCO lenses displayed a variety of additional degenerative changes in the epithelial cortex. Intrafibrillar clefts (Figs 4B, 6). Liquefaction of lens fibres (Fig 8). Small and large globules (Figs 8, 9). Multivesicular bodies (Fig 10B). Dense bodies (Fig 10C) and vesicles, often located adjacent to or on the epithelial-cortical borders (Fig 10A). Here and there an epithelial multivesicular body was adjacent to a compressed cortical vesicle or multivesicular body on the epithelial-cortical borders (Fig 10A).

A few exo- or endocytotic vesicles opened on the epithelium (Fig 10). Similar degenerative changes as described above were observed sporadically in the anterior cortical fibre of lenses without ACSCO. In some ACSCO lenses, fragmentation of lens fibres and laminated arrangement of these structures were seen in the subepithelial cortex, probably densely packed plasma membranes from lysed fibres as described by Dilley et al. 1966 (Fig 11).

In both categories of lenses, desmosome-like junctional complexes were seen on epithelial-cortical borders (Fig 12).



Fig 8

ACSCON immature cataract. Liquefied lens fibre with globules (Gl) and partially
 fibres (arrows) d = epithelial dense bodies V = epithelial vacuole e = epithelial
 Lab No 1214 77 TEM 943 (x 9 900)

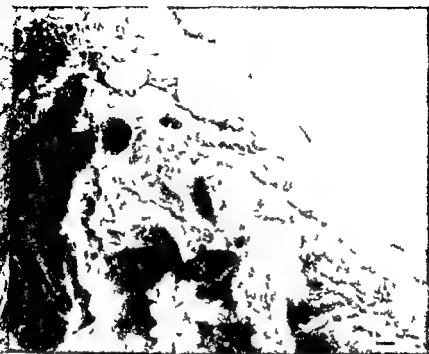


Fig 3

Picture of globules in cortex of immature cataract with ACSCO Lab No 1224/77 SEM 248 ($\times 3\,900$)

DISCUSSION

It is probable that the histopathological basis of ACSCO is the subepithelial changes clearly seen in ACSCO lenses at the light microscopical level. The subepithelial vacuoles are probably not of great importance for the biomicroscopical appearance of ACSCO since they were more often than not of the same size and number in lenses with and without ACSCO. Additional epithelial and all capsular changes were seen only at the electron microscopic level. Consequently we doubt if these changes are visible at the biomicroscopical level. However, an association between increased light scattering due to these ultrastructurally visible phenomena and the biomicroscopical appearance of ACSCO cannot be totally excluded. The finding that the subepithelial fibres of ACSCO lenses were of the deep type calls for an explanation. We suggest two possibilities: a) break-down of superficial cortex fibres and removal of the decomposed material through the capsule into the aqueous or b) formation of degenerated lens fibres possibly from the equatorial cells. We support the first — for the following reasons.



Fig 10

Cortical exo endocytotic vesicle on the cortico-epithelial border (arrow) Immature lens with ACSCO Lab No 313 77 TEM 199 ($\times 15\ 000$)

Fig 11

Laminated pattern of possibly collapsed lens fibres (asterisks) in a totally opaque area. Nucleus distorted by vacuole \square = nucleus Lab No 153/18 TEM 377 ($\times 40\ 000$)

- 1) Intra and extracellular lysosomal hydrolases have been demonstrated in the epithelium and the anterior cortex and seen as responsible for the breakdown of the epithelium and cortex in rat (Gorthy 1978) and human (Francos & Troncoso 1978) cataractous lenses
- 2) Break down of subepithelial lens fibres and epithelial cell tissue observed in place in ACSCO as indicated by the occurrence of large intercellular spaces



Fig 12

mitochondria like junctions between epithelium and cortex (arrows) and between epithelial cells (double arrow) d = epithelial dense body Immature cataract without ACSCO Lab No 1198 77 TEM 93° (x 95 800)

vacuoles with cytoplasm being detached from the wall as well as by the disintegration, liquefaction and collapse of subepithelial cortex fibres.

3) The system described by Gorthy et al. (1971) and Lénakar et al. (1972) for transporting decomposed lens fibre material via the epithelium via the epithelium and cortex. This system comprises multivesicular bodies, desmosomes and vesicles.

4) The transport vesicles and multivesicular bodies in ACSCO lenses were located adjacent to or on the epithelio-cortical borders. Some vesicles and multivesicular bodies were observed opposite each other on either side of the border, as an exchange of material from cortex to epithelium or vice versa appears possible.

5) The numerous mitochondria in ACSCO lenses indicate the possibility of a mitochondrial (oxidative) metabolism in ACSCO lens epithelium to produce energy necessary for the transport.

6) ACSCO as well as posterior subcapsular cataract is associated with lens growth to a degree that cannot be accounted for solely on the basis of cessation of lens growth (Bruun Laursen & Fledelius 1979).

7) ACSCO in immature cataractous lenses is associated with a slight but significant decrease in whole lens water percentage (Klauber & Bruun Laursen 1977). This is to be expected if cortex material which is richer in water than the nucleus (Fisher 1977; Rink 1978) leaked out of the lens and causing a decreasing proportion of the lens substance.

8) In the aqueous of human eyes with senile cortical cataract, the α and β crystallins have been found to occur in increasing concentrations as compared with non cataractous eyes (Sandberg & Closs 1979).

Having regard to the present results, we assume the histopathological basis of biomicroscopical opacity so far called ACSCO to consist mainly of subepithelial degenerative cortical changes. We therefore at present find the term (anterior) subcapsular opacity more appropriate than ACSCO although on microscopically visible capsular inclusions were pronounced in the late phase ACSCO lenses, viz. in totally opaque lenses. We had no impression of a qualitative difference as for capsular inclusions in immature cataractous lenses without ACSCO but on the other hand we cannot rule out a gradual increase in capsular inclusions as ACSCO propagates – particularly since we consider the opacity the final stage of ACSCO development.

Hypothetically, the decomposition of the superficial cortex fibres may be due to an influx of lysosomal hydrolase activators or outflow of inactivators through the lens membranes. Capsular inclusions, epithelial vacuolization, disintegrating subepithelial cortex fibres may be phenomena associated with an increasing permeability of the functional lens membranes. The decomposed material may pass via epithelium and lens capsule into the aqueous humour.

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HUMAN SENILE CATARACT AND Na K ATPase ACTIVITY IN THE ANTERIOR LENS STRUCTURES

with special reference to anterior capsular/subcapsular opacity

BY

A BRUUN LAURSEN A KLAUBER and O A JENSEN

Location and *in vitro* determination of Na K ATPase activity in the anterior structures of individual human lenses with senile cataract are reported with special reference to anterior capsular/subcapsular opacity (ACSCO)

Histochemically ATPase reaction products were found exclusively at the epithelium. Even totally opaque lenses showed strong positive reaction. Biochemically increasing ratios of Na^+/K^+ concentrations in the assay medium resulted in an increase in enzyme activity to a limited degree whereafter the activity remained stable.

We cannot decide whether the Na K ATPase activity of the anterior lens structures is unchanged in relation to ACSCO as indicated by our figures. There are methodological problems although our analytical error expressed as the variation coefficient for slaughterhouse pig lenses seems to be one of the lowest so far reported in the literature on interindividual non-pooled material.

Key words: sodium potassium ATPase – epithelium of the lens cataract human senile – biomicroscopy – histochemistry – biochemistry

Na K ATPase (sodium potassium activated adenosine 5'-triphosphatase) is associated with the active cation pump which transports Na^+ out of and K^+ into (Skou 1965) and the lens (Bonting 1965). This cation pump is active on the anterior lens surface and is located chiefly in the epithelium (Harris & Becker 1965; Kinsey & Reddy 1965). The pump is particularly active in the anterior central disc (Becker & Cotlier 1962).

a K ATPase is specifically inhibited by ouabain (Skou 1960). By means of autoradiography the enzyme has been located *biochemically* primarily in lens epithelium (Neville et al 1978) and low activities have been found in cortex and subcapsular region (Bonung 1963; Neville et al 1978). *Histochemically* Palva & Palkama (1974) using the Wachstein-Meisel method, the specificity of which has been under discussion (Firth 1978), found rat lens Na K ATPase activity between adjacent epithelial cells and on some elongating equatorial fibres, as also did Unakar & Tsui (1978) using the method of Ernst. In addition these latter authors found Na K ATPase activity in the epithelial membrane in juxtaposition with the cortex as well as in the membranes of the anterior polar lens fibres.

The aim of the present study was to examine a possible correlation between the Na K ATPase activity in the anterior lens structures and the extent of the microscopically visible anterior capsular/subcapsular opacity (ACSCO) (Bruun Laursen 1976) previously found to be an indicator of increasing $C_N + C_K$ ratio in tap water (Klauber & Bruun Laursen 1977). The study was carried out by a) gauging the histochemical location of ATPase reaction products in the anterior part of the lens; b) biochemical analysis of the enzyme activity in the anterior lens tissue; and c) examining the influence of changes in the $C_N + C_K$ ratio on the activity of the enzyme system.

Material

The study comprised 68 human lenses with uncomplicated senile cataracts as well as 94 lenses from 12 pigs (slaughterhouse material). *Histochemically* 34 lenses were studied. The material consisted of 16 specimens of anterior lens structures (capsule and adjacent lens tissue) from patients aged 62-89 years. Eight of these lenses had 0-10% ACSCO and six had 100% ACSCO (Two lenses were lost during incubation). Further, the anterior structures of 18 lenses from 61-87 years-old patients were examined.

The *biochemical* part comprised the remaining 34 lenses from patients ranging in age from 62-89 years. Enzyme activity was determined in the anterior tissues of 30 and in the posterior structures of 4 cataractous lenses. Cataractous lenses were selected and classified as described by Bruun Laursen (1976). However, noradrenaline and phenylephrine were not used and the patients were not fasted.

The following chemicals of analytical grade were used in the analyses: disodium hydrogen phosphate (Merck), EGTA (ethylene glycol bis-(β -aminoethyl)-ether N,N' -tetraacetic acid) (Boehringer Mannheim), bovine albumin (Sigma), ouabain (Sigma), gelatin (Difco-laboratories). In the following, water is aqua redestillata sterilisata adjusted to pH 7.0-8.0 by 1 drop of 0.1 N NaOH to 10 ml H₂O. All other reagents used were analytical grade. Na^+ , K^+ and Mg^{2+} were added as chlorides.

The solutions used in the *histochemical* examinations were according to Palva & Palkama (1974) except for a 4% solution of formaldehyde in an 0.2 M tris-HCl buffer, pH = 7.2 being used for postfixation. The following solutions were used in the *biochemical* investigations: 1)

um reagent 4.4% of ammonium heptamolybdate in 3 N sulphuric acid 2) 2.5% of
 in water 3) imidazole HCl buffer 50 mM pH = 7.5 For the composition of blank and
 media various chemicals were added to the homogenates (cf Methods) 4) phosphate
 in hydrogen phosphate (various concentrations) in a 12% solution of albumin in
 5) TCA reagent 7% of ferroammonium sulphate in 2.5% of trichloroacetic acid

Methods

Chemical demonstration of ATPase activity

Immediately after cataract extraction the lens capsule was opened by a cross section in the
 anterior surface. The posterior cortex and nucleus were removed before incubation.
 The lenses were also incubated. Incubation was carried out by means of the Wachstein
 method modified by Palva & Palkama (1974) except for the above mentioned
 incubation for 10 min. The incubated lens material or lens was transferred to Ames OCT
 medium and cut into 5 µm sections in an American Optical cryostat at -30°C. The sections
 were examined unmounted or mounted with glycerin. They were studied and photographed
 with an orthomat microscope using Agfachrome 50 L professional film. Copies were
 made six times. The peripheral site of the cryo-application was excluded from
 microscopy.

Chemical procedure

Cryo-extraction the site of the cryo-application was marked. The lens was placed on ice
 on the anterior surface upwards and immediately transported to the laboratory where it
 was used in imidazole buffer. One drop of gelaun (prepared the same day) was applied to
 the anterior lens surface and equator to reinforce these sites. When the gelaun was
 gently hard a 7 mm cylinder (corresponding to 38.5 mm²) of the anterior structures was
 removed and sliced under the dissection microscope. The specimen (weight 2.5-5.3 mg) -
 consisting of hardened gelaun, lens capsule, epithelium and anterior cortex - had a thickness
 of 1 mm. It was ground for 3 min in 500 µl of water in a Potter Elvehjem homogenizer on
 ice and incubation followed - in a modification after Skou (1957, 1965). The
 homogenate was transferred to a vial and stored at -80°C until used. After thawing and
 adding 50 µl aliquots were incubated for exactly 60 min at 37°C in disposable plastic
 tubes with 30 µl imidazole buffer and 50 µl enriched medium. Blank tubes contained
 nothing in addition. The final concentrations in the assay and blank media were: imidazole 30
 mM ATP 3 mM (disodium salt, the sodium being included in the total C_{Na^+} of the medium
 136 mM K^+ 90 mM Mg^{2+} 4 mM. In addition the blank tubes contained 1 mM ouabain.

Fig 1

ATPase reaction products in the epithelium of human senile immature cataractous lens
 out ACSCO. The black reaction products are seen in the epithelium exclusively
 by the Wachstein Meisel Method. Lab no 332/78 (× 400). B Location of ATPase reaction
 products at cell borders of epithelial cells in an immature senile cataractous whole-lens
 out ACSCO. Wachstein Meisel Method. Lab no 75/8 (× 540). C Epithelial cells from
 the opaque lens with abundant ATPase reaction products particularly at cell borders.
 Tangential section. Wachstein Meisel Method. Lab no 319/8 (× 400).

The protein concentration was approximately 0.3–0.6% (calculated from dry weight protein percentage – Klauber & Bruun-Laurén 1977). Furthermore, the activity of the Na⁺K⁺ATPase in the anterior structures of each individual lens was determined following C_{Na^+}/C_{K^+} ratios in the assay medium 6:150:36:190:817:5 meq/l.

The reaction was halted after 60 min by addition of 200 µl TCA. The samples were coloured with ammonium reagent (volumes 10:1) and incubated at room temperature for 10 min. Absorbance at 660 nm of samples and blanks were recorded in a double-beam spectrophotometer. The concentrations of inorganic phosphate split off from ATP were calculated from a standard curve and given as nmol P/mm² anterior lens. Standard curves were prepared every 2–3 weeks. The equations of the standard curves were calculated by means of regression analysis. The slope varied between 0.41 and 0.49, intercepts never differed significantly from 0. Daily phosphate standards were used. The median difference between 30 duplicated determinations of cataractous ATPase activity at the C_{Na^+}/C_{K^+} ratio of 136:90 was 0.4 nmol P/min² and the 0.2–0.8 nmol P/mm² (cf Fig. 2).

The pig eyes were stored on ice, the corneas and irides cut away and the lenses cut into halves. A drop of gelatin was placed on the lens. The lenses were trephined with a 7 mm trephine. The posterior capsule and the vitreous body were removed. The lenses were weighed (48–177 mg) and homogenized in 1000 µl of water and further processed as described above (C_{Na^+}/C_{K^+} ratio 136:90 meq/l).

The correlation between corresponding values of enzyme activity and the extent of cataract was calculated by means of the Spearman rank correlation analysis. Comparison groups were carried out by means of the Mann-Whitney test.

Results

High stoichiometric localization of ATPase reaction products in the anterior lens structure. The dark brown reaction products of the method used occurred in small amounts exclusively in the epithelium (Figs 1A and B). Epithelium of opaque lenses showed as a rule strong positive reactions (Fig. 1C). The reaction products were located as tiny granules in the cytoplasm, but particularly at the cell borders (Figs 1B and C). The reaction could not be inhibited by means of 1 mM ouabain. The precipitate therefore probably included more than the sensitive ATPase reaction product (such as Mg²⁺ and Ca²⁺ + ATPase reaction products). The possible occurrence of reaction products in the lens nuclei and posterior cortex was not examined in this study.

Specific biochemical Na⁺K⁺ATPase activity determinations

No significant change in the enzyme activity was found as ACSCO. Totally opaque lenses revealed activities comparable to those of slightly opaque lenses without ACSCO (Fig. 2), which indicates that the Na⁺K⁺ATPase activity is independent of cataract type and density. The median Na⁺K⁺ATPase activity in 30 cataractous lens samples at the C_{Na^+}/C_{K^+} ratio of 136:90 was 18.1 nmol P/mm² h in anterior lens structures/h and the range was 2.4–42.6. In comparison, the median activity of the 24 pig lenses was 61 nmol P/mm² h, the range being 33–90 (cf

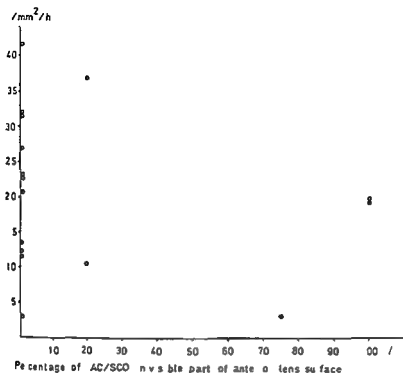


Fig. 2

Na K ATPase activity in the anterior lens structures as a function of the extent of AC/SCO. The value represents the mean of two determinations. The enzyme activity is given as $\mu\text{mol P/g/h}$ and $C_{Na} + C_{K}$ was 156.20 meq/l . $P > 0.01$ for immature cataractous lenses; \bullet totally opaque lenses.

ant value was excluded). The range was larger for the pig lenses when Na K ATPase activity was expressed on wet weight basis ($0.86-3.06 \mu\text{mol P/g/h}$ median 1.0). No significant difference was found between intra- and interindividual variation of lens pairs from 12 pigs.

Na K ATPase activities in 7 mm discs of posterior structures of 4 human cataractous lenses varied between 0 and $0.2 \text{ nmol mm}^{-2}\text{h}^{-1}$.

Effect of increasing $C_{Na} + C_{K}$ ratio of the medium on the activity of Na K ATPase in human lens structures

Increasing $C_{Na} + C_{K}$ ratios from 6150 to the level of 81.75 meq/l resulted in a significant ($P < 0.01$) increase in Na K ATPase activity (Fig. 3). Additional increase in sodium concentration and reduction in the potassium concentration did not further increase the median Na K ATPase activity. Differences in activity between

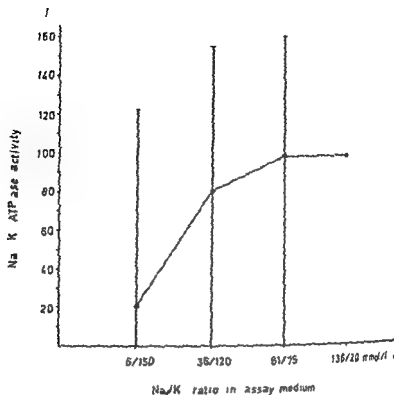


Fig. 3

Na/K ATPase activity in the anterior lens structures as a function of the $C_v + C_s$ the assay medium. The activity found at the ratio of 136/20 was given the value 1. This point shows the median value — the bars represent the ranges. The C_v values corresponding to the $C_v + C_s$ ratios of 96/120 and 81/75 were derived from a lens specimen with a activity of 2.4 nmol P per mm² and had at the ratio of 136/20 a value of 1.

groups of lenses with and without ACSCO was insignificant at all $C_v + C_s$ examined.

Discussion

In the present study the Na/K ATPase activity was expressed in relative constant biomicroscopically visible area of the anterior lens surface following reasons: 1) Histochemical ATPase reaction products of the same structures were found to be located in the epithelium only; 2) In addition, in lenses the Na/K ATPase activity range was smaller when expressed in the anterior lens tissue than on wet weight basis; 3) Furthermore the measured in the posterior lens structures of cataractous lenses were not

is not consider it expedient to express lens Na K ATPase activity in relation to lens protein firstly because it appears to be inexpedient to express the activity in relation to structures where the enzyme does not seem to lodge (cortex) and secondly because protein percentage and solubility changes with cataract development (Mach 1963 Maraini & Mangini 1973). 3) The central lens structures were chosen for Na K ATPase activity assay because cation transport appears to be most active here (Becker & Cotlier 1962).

$C_N + C_K +$ ratio of ACSCO lenses increases with increasing extent of cataract (Klauber & Bruun Laursen 1977). As indicated by Duncan (1973) such a change may be due to 1) decreasing lenticular Na K ATPase activity or 2) increasing permeability of the ion restricting lens membranes. In the present material we have tried to investigate the possibility of decreasing Na K ATPase activity in relation to ACSCO. However our examinations as well as those of other authors investigating the Na K ATPase activity of cataractous lenses (Burg 1973 Gupta & Harley 1975 Nordmann & Kleithi 1976) show that Na K ATPase activities vary within large ranges. This may be due to break-down of lens cells as indicated electron microscopically by the occurrence of large vacuolar vacuoles in the epithelium of cataractous lenses with and without cataract (Jensen & Bruun Laursen - to be published). However our examinations of Na K ATPase activities in pairs of pig lenses indicate methodological sources of

Table I

Individual Na K ATPase activity ranges in tissues from normal animals. Coefficients of variation for data from the literature as well as for our pig lens material ($N = 24$).

Authors	Year	Animal	Coefficient of variation	Na-K ATPase activity expressed per
Kinoshita	1971	mouse	ca 0.39-1.07	whole lens
Linn & Kleithi	1976	calf	0.24	g insoluble lens protein
Linn & Kleithi	1976	cow	0.86	g insoluble lens protein
Fry et al	1975	rat	0.24-0.29	g protein of ideal epithelium
Fry et al	1975	rat	0.12	g protein of jejunal epithelium
Davis & Charney (adapted procedure)	1977	rat	0.10-0.14	g intestinal epithelium
Fry et al	1978	rabbit	0.36	lens capsule + epithelium
Stanton et al	1979	rabbit	0.74	g anterior lens tissue
Ann et al	1979	calf	ca 0.46	mg insoluble lens protein
Bruun Laursen & Jensen	1980	pig	0.25	mm anterior lens tissue

error the intraindividual variation not differing significantly from the interindividual variation. Nevertheless, our coefficient of variation for ^{24}Na pig lenses appears to be one of the lowest so far reported in the literature. The interindividual variation in non-pooled materials of clear lenses (Table 1) do not feel able to decide whether the distribution of Na^+ ATPase in relation to ACSCO (Fig. 2) is due to validity of the 0-hypothesis or to methodological errors. Even though our median activities for cataractous lenses ACSCO and with 100% ACSCO were 22.6 and 19.8 nmol P/min/h, respectively, almost identical. The methodological source of error may very well be associated with the formation of tiny vacuoles during homogenisation. Our determinations on identical lens homogenates showed very small variations (Methods). Determinations on pooled homogenates also showed much smaller variations than the intraindividual variations. Unfortunately, attempts to solve the problem of possible vacuolisation by using detergents in the procedure of homogenisation were futile since the ultracentrifugate could not be effectively resuspended.

According to Fig. 3 increasing $C_{\text{Na}} + C_{\text{K}} +$ ratio is seen in ACSCO lenses associated with increasing Na^+ ATPase activity up to a certain limit. In ACSCO lenses the Na^+ stimulated pump activity may soon reach a maximum. This is consistent with kinetic the finding of Kinsey & Hightower (1978). An important detail it is our belief that no precise information on Na^+ ATPase activity in relation to senile cataract is available at present and will not be. New methods have been developed for liberating the membrane bound Na^+ ATPase from the cell walls of the cataractous lenses.

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NEW VISUAL ACUITY TEST FOR PRE-SCHOOL CHILDREN

BY

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A new test chart was developed for the measurement of visual acuity of pre school children. The symbols of the test are circle, square, apple and house. These were so designed that each symbol measures visual acuity similarly. This feature of the test was verified experimentally. The visual acuity values measured by the individual symbols correlated highly with the visual acuity values measured with the whole test (0.82-0.86). The correlation between the visual acuity values measured repeatedly, the reliability of the new test, was found to be 0.93 for adult subjects. The new visual acuity test thus fulfils the statistical criteria of a good visual acuity test. Because both children and nurses seem to like the new test, it may be useful in the assessment of visual acuity in pre school children.

Key words: vision tests - pre-school screening of visual acuity - line acuity tests

If a dozen different tests are used for testing the visual acuity of pre-school children. In two of them, Sheridan-Gardiner and Fooks tests, the child has copies of symbols in front of him and points to the symbol he thinks is shown at the chart as a single symbol. In the Sjögren test and Snellen E test one measures both visual acuity and left-right comprehension. In picture charts the different figures measure visual acuity heterogeneously and the test requires ability to name some of the seen objects (Sheridan 1963). These charts work rather well in practice, however, and therefore we undertook a test construction on this basis.

A new visual acuity chart was developed by one of us (L. H.) for the measurement of visual acuity of 3-5 years old children. The chart was designed to allow the child to respond either by pointing or by naming, and the individual symbols were chosen so that their naming would be easy. We present here a statistical analysis of the new

Test symbols

Square and ball and their derivative apple and house were chosen to be the symbols. The symbols were drawn in several slightly different forms and blurred equally i.e. when seen with positive overcorrection or diffusing lens became similarly blurred and were eventually seen as a diffuse ring in which a cross appears in the centre.

The reliability and validity of the new test and its item analysis

The symbols were studied by using seventeen 20-35 years old adults as test subjects. Visual acuity was measured with a prototype of the new test chart and the Snellen test chart at 6 m twice binocularly and monocularly with both eyes illuminated with 10 candles/m².

Table I
Correlations between the new test and its individual test times

	Ball	Apple	Square	House
Whole test	0.86	0.82	0.86	0.84

Table I shows the correlations between the different test symbols (test times) and monocular measurements pooled ($N = 34$). The visual acuity values measured with each symbol have a high correlation with the visual acuity values measured with the whole test (0.82-0.86). The symbols thus measure visual acuity similarly and the structure of the test symbols fulfils the criteria of a good visual acuity test.

The reliability of the new test (correlation between repeated tests) was 0.64. The corresponding reliability of the Snellen E test was 0.96. Both tests when repeated thus give the same visual acuity values.

The relationship between the size of E symbol and the new symbols

This relationship was studied twice. In the earlier phase of this work we used 10 subjects and found that the new test symbols have to be 1.5 times larger than the E symbol in order to give same visual acuity (relationship 1.5 ± 0.05). Following the introduction of the visual acuity chart, several colleagues reported that the visual acuity values measured with the new chart were higher than those measured with the E chart. Therefore the correlation was studied a second time.

Thirty adult persons (20-35 years) were subjects. Fig. 1 shows the symbols used. The charts were shown at several different distances and the distance at which 25% of 100 answers were wrong was recorded.



Fig. 1

Examples of the E symbol chart and the new symbol chart used in comparing the size

threshold tasks in these two tests are different when the E symbol is blurred to the point of being barely perceptible it is seen as a dim C and the direction of E can be guessed by estimating which side of the symbol is lighter. The new symbols when blurred are recognized by the number of corners pointing either up into the dim ring a visual task quite different from the recognition of the E symbol.

The relationship between the size of the new symbols and the E symbol was now found to be 1.63 ± 0.18 . Thus the symbols of the new chart are not too large they are a fraction larger. The lower visual acuity values of the children when using the E test reflect the additional tasks involved (left-right comprehension, motor responses) unrelated to visual perception per se.

New test chart

The new chart resembles the Snellen E chart except that it was prepared for testing at 3 m. If used at 6 m the visual acuity values have to be multiplied by 2. The 3 m distance was chosen because the chart is meant for screening of visual acuity in day centres, nursery schools and during home visits and there 3 m is a more practical distance than 6 m or 9 m. It also makes communication with the child easier. The uppermost line 0.1 contains three symbols and thus functions better as a line than the regular E chart having only one 0.1 symbol (Bailey & Lovén 1976, Verman 1975, Tommila 1972). The distance between the rows is equal to the height of the symbols in the smaller row and the distance between the symbols is equal to the width of the symbol (see Fig. 2).

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PSEUDO EXFOLIATION MATERIAL

Electron Microscopy after the Application of Lanthanum as Tracer Particles and Ionic Stain

BY

MARTIN DAVANGER

Lenses with pseudo-exfoliation (PE) were suspended in a solution of lanthanum which was present partly in ionic form partly as electron-dense tracer particles in colloidal solution dependent on pH and on the concentration of lanthanum. By electron microscopy the tracer particles were found in a narrow zone above the surface of the PE excrescences. Only a few of the smallest particles had penetrated into their most superficial layer. In the specimen treated with a lanthanum the effect was an electron dense staining of the PE material within a 1.2 μm thick superficial layer. The PE fibrils as well as parts of the interfibrillar spaces were stained. These results indicate the presence of proteoglycans and confirm the concept of an interfibrillar matrix which excludes other macromolecules and particles from penetrating into the material.

Key words: pseudo exfoliation - amyloid - lanthanum - tracer particles

From a biochemical point of view the pseudo-exfoliation (PE) material is considered to consist of proteoglycosaminoglycans (proteoglycans) acid mucopolysaccharides. Morphologically the characteristic element is the PE fibril which can be seen both by transmission and by scanning electron microscopy.

The PE fibrils seem to be randomly distributed in three dimensions. They are closely packed; neighbouring fibrils are separated by a certain distance. The space between the fibrils appears empty both by transmission and by scanning electron microscopy when the specimens are prepared by conventional methods. However, by the application of tracer molecules and also by special staining methods it has been shown that the interfibrillar spaces are filled with a ground substance. It

has the remarkable property that tracer particles are totally or partially removed from the PE material (Davanger & Pedersen 1975, Davanger 1977, 1978). In the present study lanthanum has been used in the preparation of PE material for electron microscopy. Two different properties of lanthanum have been used:

gradually increasing pH of a solution of lanthanum nitrate colloidal particles lanthanum hydroxide are formed. These particles are electron-dense and they can be used as tracer particles in electron microscopical work (Revel & Karnovsky 1967).

The treatment of the PE material with lanthanum applied as a tracer may also supplement earlier experiments in which other tracer substances have been used.

In addition to the use as a colloidal tracer lanthanum has been used also in ionic form as an electron microscopical stain which binds to certain tissue components in conjunction with staining with other heavy metals (Schatzki et al. 1975). Although the staining mechanism is not well known it is assumed that ionic lanthanum has an affinity to proteoglycans and mucopolysaccharides (Behnke 1968, Shea 1971, Schatzki et al. 1977).

It should be noted that a neutralized lanthanum solution contains both ionic lanthanum and lanthanum in colloidal form (Schatzki & Newsome 1975). Lanthanum can be used mainly as a tracer or mainly as a stain dependent partly on pH of the solution and partly on the concentration of lanthanum.

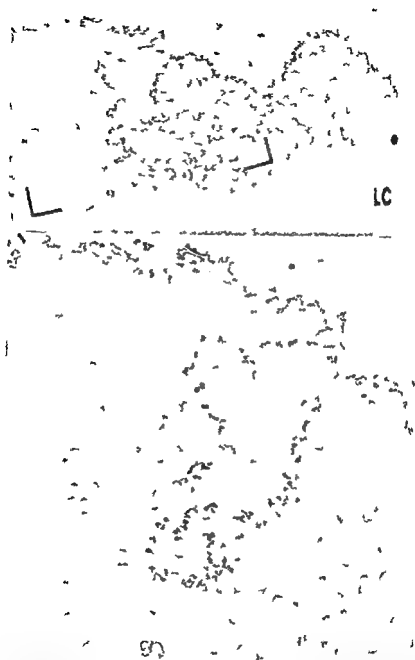
In the present paper describes some results of the application of lanthanum used as a colloidal tracer and as an ionic stain on PE material on the anterior lens surface.

Material and Methods

Lenses were removed from the peripheral band and the central disc on the anterior lens capsule and studied. The lenses were cataractous and they were obtained by cataract cryo-extraction as described by Benner & Luft (1959) prior to the operation.

The lenses were suspended directly in a solution of colloidal buffered osmium tetroxide prepared as described by Benner & Luft (1959) containing 2 to 5% lanthanum nitrate. The lenses were fixed in this solution for 2 to 24 h. Five other lenses were first suspended in a 2% solution of lanthanum nitrate to which 0.01 N NaOH had been slowly added until pH about 7.8 and a faint opacity could be observed. After 4 to 1 h the lenses were suspended in colloidal buffered solution of osmium tetroxide as described above. In these cases 1% lanthanum nitrate was added.

After fixation the lenses were dehydrated in graded ethanol solutions. Specimens were removed from the anterior lens capsule at the site of the peripheral band and the central disc. The use of the cryo-extractor was avoided. The specimens were embedded in Epon 812 and sectioned with a Reichert Ultracut microtome. Ultrathin sections were contrasted with lead citrate or an aqueous solution of uranyl acetate or both. Electron microscopy was performed with a Jeol 100 B electron microscope.



FR 1

Pseudo-exfoliation excrescences on the anterior lens surface. Lanthanum trioxide
adhere to the surface of the excrescences. The area in micrograph B is as in
micrograph A (as indicated by angles). Uncontrasted section. LC Lenticular

A x 6000 B x 1400

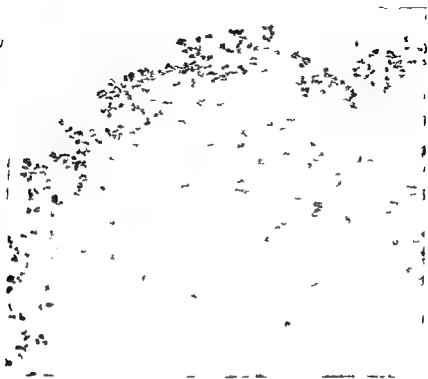


Fig 2

pseudo-exfoliation material Lanthanum tracer particles The larger particles adhere to the surface of the excrecence the smallest particles have penetrated sparsely into the superficial layer of the material Uncontrasted section $\times 43\,800$

Results

On specimens which had been treated with lanthanum at high concentration or at a pH near 7.8 the effects of lanthanum were revealed mainly by the presence of distinct lanthanum tracer particles while ionic staining was less obvious. The particles were also electron-dense in uncontrasted sections and micrographs in uncontrasted sections were particularly suited for the demonstration of the tracer. Particles of different sizes were present, with diameter from about 50 nm to less than 5 nm (Figs 1 and 2). No obvious pattern was found in the distribution of the tracer particles relative to the PE excrecences. The main part of the particles and all those which were larger than 50 nm were located within a narrow zone along the surface of the PE material (Figs 1 and 2). Evidently colloidal particles of lanthanum hydroxide had adhered

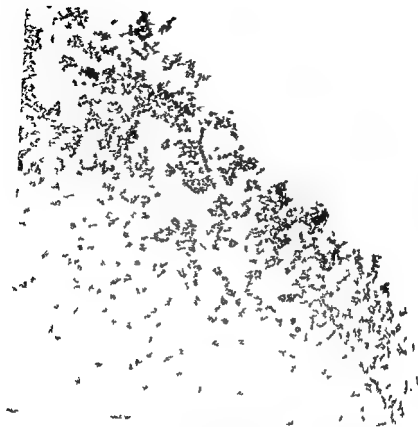


Fig 3

Pseudo-exfoliation material. The superficial layer ($1.9 \mu\text{m}$) of the excrecences is ionic lanthanum which has not penetrated into the deeper parts. Lanthanum raised $\times 9,000$

to this surface during the preparation and none of the larger particles penetrated into the PE material.

By using higher magnification it could be seen that a few small (tracer particles 5 nm) were localized in the superficial parts of the PE material (Fig 4). These particles had a tendency to be located along the PE fibrils and in the interfibrillar spaces. Except for this tendency, their distribution seemed random.

The specimens which had been treated with lanthanum at a lower concentration and/or at a lower pH demonstrated a somewhat different picture. Lanthanum tracer particles were present only to a minor degree and the few particles had a distinct appearance and form. However, in these specimens the treatment with lanthanum was revealed by lanthanum staining of parts of the PE material.

cases the presence of lanthanum is best demonstrated in uncontrasted (Fig 3)

lanthanum staining was clearly limited to superficial parts of the PE excrescences. Beyond a depth of $1.2\ \mu\text{m}$ no effects of lanthanum could be found. Degree of staining decreased gradually from the surface inwards, however the difference between stained and unstained PE material was usually quite marked.

PE fibrils were denser and thicker in the stained parts of the material and had a more ragged outline as compared with the fibrils of the unstained areas. In unstained electron lucent areas between the fibrils were reduced in size. Near surface of the excrescences most of the material was stained and the fibrils could be distinguished only with difficulty. The lanthanum staining was not homogeneous and by higher magnification it could be seen that the staining consisted of numerous small dense, more or less confluent spots of irregular form.

In some of the specimens the staining with lanthanum did not reveal further information about the ultrastructure of the PE material. However in other specimens details concerning subfilaments and crossbands of the fibrils appeared in the PE excrescences. This will be dealt with in a subsequent paper.

Discussion

Electron micrographs of PE material which has been prepared by conventional methods. The interfibrillar spaces do not appear to contain any material. However distribution of the lanthanum tracer particles as found in this work and shown in Figs 1 and 2 demonstrates beyond doubt that the interfibrillar spaces are filled with a substance which prevents most of the tracer particles from entering the PE material. The concept of an interfibrillar matrix as part of the PE material (Davanger & Pedersen 1975; Davanger 1977, 1978) is thereby confirmed. This matrix is usually electron lucent and invisible by electron microscopy. While the main part of the lanthanum tracer particles are excluded from the PE material, particles with a diameter less than about $5\ \text{nm}$ are sparsely admitted into the superficial parts (Fig 2). Accordingly this size seems to represent the limit of exclusion of lanthanum tracer particles from the PE material. In this connection it should be noted that the molecular tracer horseradish peroxidase with a molecular diameter of about $4.5\ \text{nm}$ (Vege et al 1971) was excluded from the material (Davanger & Pedersen 1975) while the smaller tracer microperoxidase (diameter about $2\ \text{nm}$ (Feder 1971)) was admitted only into its superficial parts (Davanger 1978). It may be presumed that tracers are totally or partially excluded partly for

sterical reasons but that also other factors such as electrostatic forces are involved.

According to several authors (Behnke 1968, Shea 1971, Johnson 1977) lanthanum has affinity to proteoglycans and mucopolysaccharides. The staining with lanthanum of the superficial parts of the PE material demonstrates and supports the concept that the material consists of or contains such substances.

It has been reported that lanthanum staining is found at or near the surface of tissue fragments while the deeper layers remain unstained (Lesseps 1967). The absence of lanthanum staining beyond the surface layers has been considered caused by poor penetration of lanthanum ions in most tissues. In the present study, lanthanum staining was confined to the superficial 1-2 μm of the PE material. Obviously, the penetration of lanthanum ions is very restricted in the PE material and more restricted than in most tissues. This is another demonstration of its penetrability as one of the characteristics of the PE material.

Poor penetrability of lanthanum has also been noted for amniotic epithelium (Sørensen & Bari 1968). Accordingly, the partial exclusion of lanthanum is one of the properties which the PE material has in common with amniotic epithelium.

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ON THE ULTRASTRUCTURE AND THE FORMATION OF PSEUDO EXFOLIATION MATERIAL

BY

MARTIN DAVANGER

Pseudo-exfoliation (PE) excrescences on the anterior lens surface were studied by electron microscopy. In some cases the superficial layer of the excrescence consisted of a meshwork of fibrils and filamentous subfibrillar units. It could be clearly seen that the fibrils were formed by lateral aggregation of filamentous units. Both the filaments and the fibrils had crossbands regularly spaced at 17 nm. In some fibrils every third crossband was fainter than the neighbouring crossbands. Thereby a secondary periodicity appeared, the period consisting of three crossbands in the order dense-faint-dense. The length of this period was $17 \times 3 = 51$ nm, i.e. the same as the distance between the crossbands of conventional PE fibrils. All transitional forms between the fibrils with 17 nm periodicity and the conventional PE fibrils were found, so there was a gradual transition between the material described and conventional PE material. It is suggested that the material on the surface of the excrescences represent newly formed PE material. This may be formed by condensation and linear polymerization of two kinds of units corresponding to the dense and the faint crossbands.

Key words: pseudo-exfoliation — fibrils — filaments — crossbands.

When examined by electron microscopy the pseudo-exfoliation (PE) fibrils constitute the characteristic element of the PE material. Several authors have shown that these fibrils are composed of filamentous subunits (Ringdahl & Hultén 1977; Davanger 1977; Davanger & Hovig 1978; Dark et al. 1977) but a more detailed demonstration and description of these subunits has not been given. Evidence has been presented indicating that linear filamentous units are also present in the interfibrillar spaces and it has been suggested that these filaments are of the same kind as those constituting the PE fibrils (Davanger 1977, 1978).

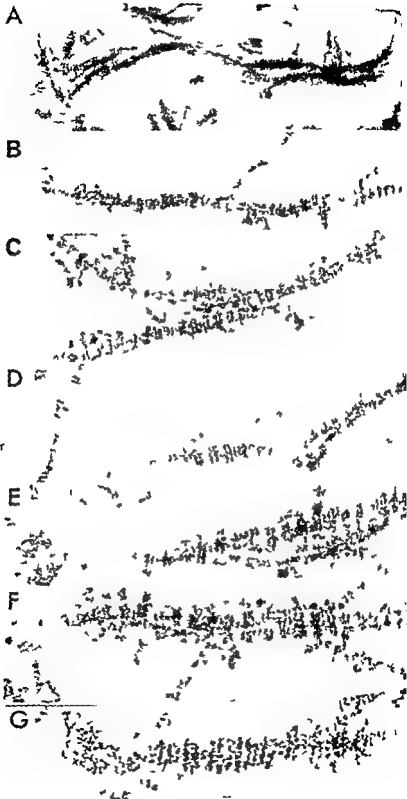
Received January 17th 1980



Fig 1

Network of filaments and fibrils at the superficial layer of pseudo-exfoliation excrescences. Fibrils are obviously formed by lateral aggregation of the filaments. Both the filaments and the fibrils have a 17 nm periodicity. Section contrasted with lead and uranyl. λ 36 600

Several authors have described crossbands as a feature of the PE fibrils. Usually crossbands are faint and often difficult to discern. They present themselves only as faint periodic variations of the thickness in the fibrils. However, as the fine line of the fibrils usually appears rather blurred and woollen, the crossbanding is clearly seen in most fibrils. The distance between the crossbands has been measured to 51.56 nm (Bertelsen et al 1964), 40.56 nm (Ringvold 1969), 50.55 nm (Dunn 1970), 30 nm (Davanger 1977, Davanger & Hovig 1978) or 20 nm (Dark et al 1977). Dunn's electron microscopy of PE material which had been stained en bloc with



lanthanum it was observed that in parts of the PE excrescences filamentous fibrillar units were clearly visible. The filaments were found also in the fibrillar spaces and it could be clearly seen that the fibrils were composed of filamentous units. Further the study of these specimens revealed new details of the texture and the crossbanding of the PE fibrils. In the present paper these findings will be demonstrated, described and discussed.

Material and Methods

The material consists of five lenses with PE. The lenses were cataractous and they were obtained by cataract cryo-extraction. The lenses were suspended in a solution of sodium chloride buffered osmium tetroxide containing 2 to 3% lanthanum nitrate. Results of the electron microscopical examination of these lenses have been reported from another point of view in a recent publication (Davanger 1980). Details of the preparation and methods of examination are described in that paper.

Results

PE excrescences consisted mainly of PE material with the conventional appearance. Most of the surface layer of the excrescences was stained by lanthanum as it has been described in a recent paper (Davanger 1980). However in one of the five lenses the material had a different and typical appearance in parts of the PE excrescences, mainly at their surface and especially near their apices. In the following the morphology of these parts of the excrescences will be described. The electron microscopical picture (Fig. 1) was dominated by two distinct and characteristic morphological elements. One of these was a filament with a uniform thickness of 10 nm and a length in the sections up to 1 μ m. These filaments were usually straight or slightly bent. The filaments had an obvious periodic construction with alternating dark and light zones of about equal length. The repeat period consisted accordingly of one dark and one light zone. This period was 17 nm long. The other morphological element was a fibril with marked and characteristic crossbands. Although these fibrils had no longitudinal striation they were obviously formed by lateral aggregation of the filaments recently described. Several features support this concept. Some filaments were part of a fibril for only a portion of their

Fig. 2

Fibrils in the superficial layer of pseudo-exfoliation excrescences. Crossbands 17 nm apart arranged in the order dense-faint-dense-dense-faint-dense etc. Secondary periodicity of 17 \times 3 = 51 nm. Sections contrasted with lead and uranium. A \times 43 100 B \times 53 000 C \times 54 000 D \times 52 000 E \times 79 000 F \times 84 000 G \times 118 000

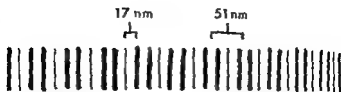


Fig 3

Schematic drawing of a pseudo-exfoliation fibril. Crossbands 1: nm λ γ periodicity. The crossbands are arranged in the order dense-faint-dense-dense-faint etc. A secondary period of $17 \text{ nm} \times 3 = 51 \text{ nm}$ contains one dense, one faint and one crossband.

length. One filament might be entangled with more than one fibril, where irregular meshwork was formed, in which the fibrils formed the crossbands. At the site of junction between a fibril and a filament, it could be seen that the filament was added to the thickness of the fibril. As will be described in the following, filaments and the fibrils had the same periodicity.

These fibrils were randomly distributed in relation to each other, except a tendency to form irregular groups in which the individual fibrils were incompletely separated. The length of the fibrils, as seen in the sections, was 1 to $2 \mu\text{m}$. Also the thickness varied, a typical diameter being 50 nm .

The fibrils concerned were conspicuously crossbanded, with alternating dark and light lines crossing the fibrils at a right angle. The basic repeat period was the same as the period of the filaments in the spaces between the fibrils. At the site of junction between a fibril and a filament, the dark and light zones of the fibril were aligned in phase with the alternating dark and light crossbands of the filament. Obviously, these crossbands were the result of the alignment of the alternating dark and light zones of the filaments of which the fibrils were composed.

A closer examination revealed that in many fibrils the density of the crossbands varied in a systematic manner. Every third crossband was slightly or even fainter than the adjoining crossbands (Fig. 2). Accordingly, the crossbands were arranged in the order dense-faint-dense-dense-faint-dense etc. A secondary periodicity appeared, consisting of three of the original periods, that is, the length of the new period was $17 \times 3 = 51 \text{ nm}$ (Fig. 3). This is the same as the period length of conventional PE fibrils.

In those fibrils where the 51 nm periodicity was dominant, the dense crossbands in each period tended to be longer than the faint crossbands; accordingly, the dense crossbands protruded slightly beyond the general level of the fibril. In some of those fibrils, the white lines separating two neighboring dark crossbands were thin, irregular and not easily seen. The appearance of the fibrils was not much different from the appearance of the conventional PE fibrils.

neighbouring dense crossbands tended to fuse to form one crossband which was mainly as a thickening of the fibril. Such thickenings were regularly spaced 17 nm.

According to the above, all transitional forms could be found between the fibrils with 17 nm periodicity and the conventional PE fibrils with 51 nm periodicity. Comparing with this, there was usually no sharp boundary between the conventional material and the material consisting of a meshwork of filaments and fibrils with 17 nm periodicity as described above.

Discussion

Treatment with lanthanum may have enhanced the features described in this study and made them more conspicuous, but lanthanum is probably not a condition for their demonstration. The presence of filaments as well as fibrils in the PE material has been described before. Likewise, the coalescence of filaments into PE fibrils has been presumed or demonstrated by several authors (Ringvold & Husby 1977, 1978a,b; Dark et al. 1978). However, a detailed demonstration of the process has not been presented before.

Some of the fibrils shown in the present paper have many characteristics in common with the fibrils found by Ringvold (1970a) by negative staining of PE material. This concerns also the crossbands and the periodicity. It should be noted that fibrils remarkably like those described above have been found by Ringvold (1970b) in sections of iris tissue from old patients operated for senile cataract. No clear signs of the PE syndrome had not been found in these patients.

The meshwork of filaments and fibrils on which the present work has concentrated is clearly a part of the PE excrescences and the PE material, although its appearance differs in details from that conventionally described for PE material. The relationship between the conventional PE fibrils (period length 51 nm) and the material with a 17 nm periodicity is underlined by the presence of transitional forms between the two types.

It is reasonable to look at this transition from a dynamical point of view, that is, to consider the different morphology at different locations of the PE excrescences as representing different stages in the formation of the PE fibrils and the PE material.

4) The PE material may be thought to be formed by a gradual condensation of the anterior lens surface and other surfaces of substances which have been released in the aqueous. The excrescences must be presumed to increase in size by accumulation of new material on their surface. The material which has been demonstrated is thought to represent PE material in an early stage of formation.

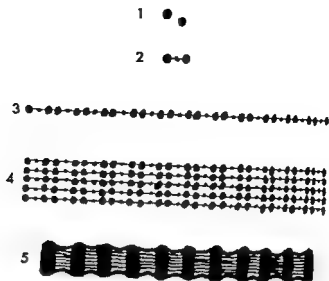


Fig. 4

Formation of a pseudo exfoliation fibril (hypothetical) 1 A solution of two molecular units, dense and faint 2 Two dense and one faint unit combine to form a monomer of the fibril 3 Monomers combine end to end to form a filament. The filaments are seen regularly spaced at 17 nm 4 Immature PE fibrils are formed by lateral association of filaments. Crossbands (dense and faint) are formed 17 nm apart, by the alignment of basic units 5 Mature PE fibril. The fusion of two adjacent dense crossbands results in the disappearance of the faint crossbands, producing a periodicity of $17 \text{ nm} \times 3 = 51 \text{ nm}$.

Although the real process may be different, it is tempting to demonstrate in simple and schematic form a possible mechanism for the formation of the fibrils (Fig. 4). The basic units may be of two kinds, of which one will develop into dense crossbands and the other into the faint crossbands. The two different kinds of units may be thought to combine to form a linear symmetrical block consisting of one dense, one faint and one dense unit. This block is the monomer from which the fibrils are formed by polymerization. The monomers attach to each other end to end, thereby forming a filament. Several filaments combine by lateral association to form bundles. During this process the basic units align to form the crossbands of the primary fibrils, 17 nm apart. This primary periodicity changes gradually to the 51 nm periodicity as described earlier. At the same time the original thick material condenses into the conventional PE fibril, and the electron-lucent zones between the original crossbands disappear.

The immature PE material, characterized by the presence of filaments and a periodicity, is formed at the surface of the PE excrescences. The transformation of this material into conventional PE material may be thought of as a maturation process.

may be analogous with the aging process which takes place during the first after the formation by polymerization of collagen fibrils (Gross 1961). The action of collagen has been explained by increasing perfection of fit between subunits as they gradually pack together. As in the case of collagen the aged maturation process of PE material seems to lead to the formation of a and insoluble material.

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INDOCYANINE GREEN FLUORESCENCE ANGIOGRAPHY

BY

LEA HYVARINEN¹ and ROBERT W. FLOWER

Indocyanine green (ICG) fluorescence angiography has been further refined for use in both laboratory and clinical investigations. In the present modification of the Zeiss fundus camera all lenses except the aspherical objective lens have been specially antireflection coated to increase light transmission in the spectral region around 800 nm. A 300 watt iodine iodide lamp corner as light source has replaced the conventional xenon flash lamp. This light source produces a retinal irradiance of 265 mW and therefore restricts retinal exposure time to 11.9 seconds but that time is more than adequate to record passage of dye through the choroid. Spatial resolution of the fundus on the film has been increased from 11.7 microns to 7.4 microns.

With these technical refinements the choroidal circulation can be studied at frames per second which is adequate to document the very rapid movement of blood through the vasculature. ICG angiography may change our interpretations of choroidal circulatory phenomena which are now based on fluorescein angiography and it clearly is an effective tool in laboratory (experimental) investigations.

Keywords: indocyanine green fluorescence - angiographic studies of the retinal choroidal circulation.

Sodium fluorescein angiography has been an important part of ophthalmic armamentarium for well over a decade now and in clinical diagnosis of retinal vascular diseases cannot be impugned. However its usefulness in studying bloodflow dynamics is limited especially in the choroid where transmittance of visible light wavelengths is poor and extravasation of fluorescein dye readily occurs.

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are even instances in which misinterpretation of fluorescein angiograms may lead to erroneous conclusions about choroidal bloodflow (Flower 1972 1980). Development of indocyanine green (ICG) fluorescence angiography was initially aimed solely as a way to obviate the limitations imposed by fluorescein dye during experimental studies of choroidal bloodflow dynamics. It was serendipitous that in the development of the technique it became evident that ICG angiography can be used safely and routinely on human subjects as well. Clinical ICG angiography has been performed since about late 1973 and the data obtained have been invaluable to refinement of the technique. The accumulated angiographic material is still insufficient to warrant interpretation of choroidal circulatory phenomena in pathological conditions. However some observations can be reported.

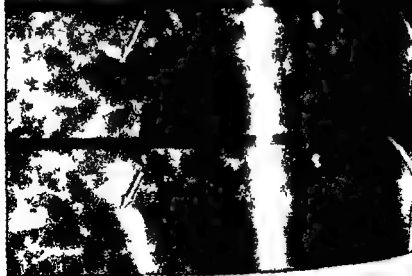
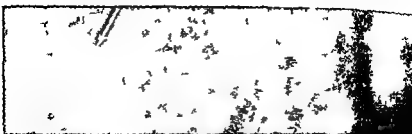
Both ICG absorption angiography and the early method of fluorescence choroidal angiography have been described in detail before (Flower 1972a,b Flower &heimer 1973 1976 Flower 1976) therefore it is the refinements and resulting improvements in spatial and temporal resolution achieved since these which are described below.

Technique

Following the development of the new camera for ICG angiography approximately 100 ICG human angiograms were made at the University of Oulu Department of Ophthalmology and 480 in the Wilmer Ophthalmological Institute. ICG and fluorescein angiograms were done a few minutes apart. Injection of ICG dye was made via a 3-way cannula followed immediately by a saline flush (Flower 1973). No gastric or vasovagal reactions occurred and none of the patients were discomforted by the illumination used for ICG near infrared photography.

ICG angiograms of rhesus monkeys were made at the Wilmer Ophthalmological Institute. The injection technique was identical with that in human beings except that smaller volumes were injected and all animals were anesthetized with halothane.

Fluorescence ICG angiography is performed in much the same way as fluorescein angiography the major difference being that ICG angiography utilizes near infrared light wavelengths while fluorescein utilizes light wavelengths in the visible portion of the spectrum. Descriptions of the absorption and emission ICG dye spectra as well as characteristics of the photographic film and excitation and barrier filters used in choroidal angiography have been previously published (Flower &heimer 1973 1976). Briefly the advantages of ICG dye in choroidal angiography are that there is no extravasation from the choriocapillaris, that it absorbs



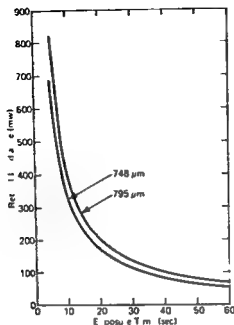


Fig 1

indicating maximum level of safe retinal irradiance as a function of exposure time

and fluoresces in a spectral region where retinal and choroidal pigments are transparent and that its long wavelengths of emitted light are more than six times less scattered by the ocular media than the shorter visible light wavelengths used by fluorescein. Its principal disadvantage is that it does not fluoresce strongly. Whereas the quantum efficiency of fluorescein dye in blood is nearly 1 and that of ICG dye is only 0.13, and if the relative fluorescence intensity of fluorescein is arbitrarily set at 1, that of ICG is 25 times less. Therefore developing the instrumentation for choroidal angiography essentially has been an exercise in increasing ability to record the fluorescent light energy emitted by ICG dye in the retinal blood vessels.

Fig 2

consecutive frames of a 20 frame per second ICG fluorescence angiogram made of an rhesus monkey. Arrows indicate the location of the same individual choriocapillaris throughout the sequence. In frames A-D the arterial feeder of the lobule can be identified. In frames D-F to the left of the arrow the drainage venules into which the lobule empties can be seen. Note that in frame A-F the lobule appears white as it fills with dye and appears black in frames G-J where it has essentially emptied. Maximum filling of the lobule occurred by frame D, indicating a filling time of only 0.5 seconds.

The lenses of the fundus camera are normally antireflection coated to transmit light throughout the visible spectrum with maximum transmission occurring at about 530 nm wavelength. However, near 800 nm wavelength ICG fluorescence occurs; the measured loss of light energy at or about 4% (the loss nominally occurring at most wavelengths when elements are used). By antireflection coating lens elements specifically at this wavelength, this loss can be reduced to as little as 0.3% per lens surface. If done to all lenses in the Zeiss fundus camera imaging optics except the objective lens which is normally uncoated. Providing one of the auxiliary lenses is not used, there are 14 lens surfaces in the fundus camera optics. At 800 nm wavelength, with the original lens coatings, total light transmission was $0.960^{14} = 0.560$ or 56%. With proper antireflection coatings, total transmission became $0.997^{14} = 0.959$ or 96% which amounts to 40% more light reaching the film exposures.

The effective aperture stop in the Zeiss camera is the hole drilled through the diagonal mirror located between the aspherical objective lens and the eyepiece wheel. Normally this hole is 5 mm in diameter, but by enlarging it to 1 cm, much more light can pass through the imaging optics to the photographic film. Reduction of this diagonal mirror surface which reflects light into the eyepiece results in an 11% decrease of 800 nm wavelength excitation light energy. This is regained by replacing the aluminum coatings on both diagonal mirrors with illumination optics with quartz-clad silver. Added to the gain in transmission achieved by using better lens coatings, a total gain of 5.7 times in light transmission at 800 nm wavelength makes it possible to photograph the fundus at the 2.5 times magnification of the standard Zeiss fundus camera.

Of equal importance is improving temporal resolution of the rapid dye filling sequence of events. Since no flash lamp light source can be recycled at a sufficiently high rate and yet deliver enough light energy to perform ICG angiography, a continuous light source was installed. A 150 watt quartz halogen lamp was used, and in order to increase the light intensity, an adjustable mirror was also installed behind the lamp. More recently, a 300 watt indium iodide lamp which has a much larger infrared component at 800 nm wavelength. Caution must be exercised in using a continuous light source to insure that retinal irradiance does not exceed safe limits. For the approximate 30 degree fundus area illuminated by the Zeiss fundus camera and for the light wavelengths required to excite ICG dye to fluoresce, the maximum permissible exposure of the retina is 3160 mw seconds (ANSI National Standard for the safe use of lasers 1973). Retinal irradiance is a function of continuous exposure time in Fig. 1. The indium iodide lamp produces a retinal irradiance of 260 mw, consequently, an exposure time

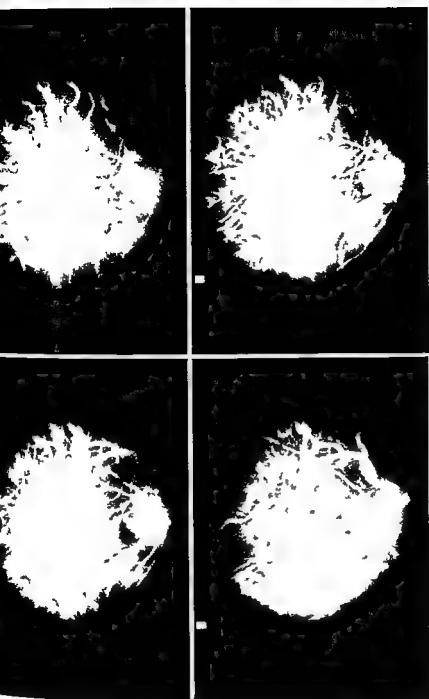


Fig 3

angiograms with unusually good visibility of both arteries (A, B) and veins of choro d (C, D)

more than 11.9 seconds is permitted but this is more than adequate to passage of dye through the choroidal circulation.

The brighter light source coupled with improved light transmissivity of the camera optics makes it easy to now record angiograms at rates up to 20 frames per second. This compares favorably with the 4 or 5 frames per second previously achieved. At the same time spatial resolution of the fundus on film was improved from 11.7 microns to 7.4 microns which is nearly equal to that achieved in fluorescein angiography.

During the development of the new camera several hundred angiograms were made to test the different improvements and the clinical application of the new system. In these angiograms a number of interesting phenomena were recorded which are reported in this paper.

Results

Animal experiments

We have recently succeeded in photographing the passage of ICG dye through the choroid at 20 frames per second. The ten consecutive frames in Fig. 4 show visualization of a sharp dye wavefront moving through the entire choroidal vasculature of an adult rhesus monkey eye following retrograde injection of less than 0.05 ml of dye into the contralateral common carotid artery. This demonstrates that the average time required for dye transit across individual choroidal lobules is on the average 1/5 second. The dye wavefront may be easily followed in the particular lobule indicated by the arrow throughout the sequence. These angiograms and others made the same way confirm and even refine choroidal bloodflow measurements made earlier. But more significantly, in the context of the present paper, they demonstrate the necessity for using high speed angiography to study the choroidal circulation. Obviously subtle changes in choroidal bloodflow could be completely missed in angiograms of less temporal or spatial resolution.

Human Studies

Although the choroid is nearly transparent to both those wavelengths of light used to excite ICG dye to fluorescence and those emitted by the dye, there is a variation in visibility of choroidal vessels among different individuals. The reasons for this variation are not apparent. Choroidal vessels were quite often not visualized in young healthy persons with no known choroidal pathology. This visibility was not due simply to poor photographic technique; resolution and contrast details was good. In less than one third of the angiograms were choroidal vessels seen as clearly as those in Fig. 3 where both the short ciliary arteries and

Fig 4

ICG is more reliable than fluorescein for measurement of dye appearance time in choroid. ICG fluorescence is also attenuated by the pigment. Note that the fluorescence intensity of dye in the cilioretinal artery (cra) is higher than in nearby choroidal vessels although dye concentrations in these vessels most probably are identical.

Later the choroidal veins are well defined, smaller vein branches and microcapillaries are not visualized.

For measurement of dye appearance time in the choroidal vasculature, ICG is more reliable than fluorescein because it is more easily seen through the retinal and choroidal pigment and it does not become extravasated. ICG fluorescence, however, can be attenuated by pigment as demonstrated in Fig 4. First appearance of dye traces in the cilioretinal artery is seen in the frame preceding Fig 4, whereas retinal vessels are barely visible. Note that the fluorescence intensity of dye in the cilioretinal artery is higher than that in the nearby choroidal vessels although dye concentration in both these vessels would be expected to be nearly identical.

Blockage of ICG fluorescence is similar to that observed with fluorescein. Blood pigment clumps in retina can produce distinct areas of reduced or non-fluorescence in angiograms. Similarly, the egg yolk substance in Best's disease blocks choroidal ICG fluorescence. But on the other hand, ICG angiography does

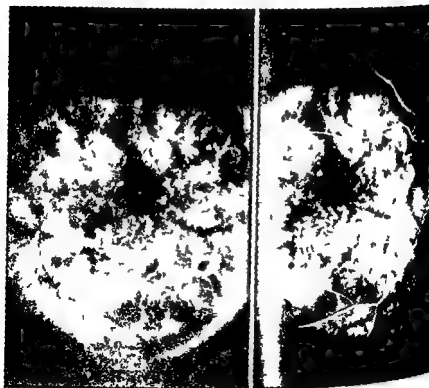


Fig 5

In ICG-angiograms (A, B) there is an area of decreased dye fluorescence (arrow) detectable in corresponding fluorescein angiograms (C, D). Times measured from appearance of the dye: 1.6 seconds (A, C); 3.7 seconds (B, D).



Fig 6

- fluorescein angiogram of a large submacular neovascularization with a large feeding artery (arrow). The draining vein wraps around the artery and its proximal part cannot be seen because of diffuse fluorescence near the disc.
- In the ICG-angiogram the vein is seen not to follow the artery to the disc margin and thus photocoagulation was placed close to the edge of the disc.

times reveal circulatory changes in choroid which are not detectable in corresponding fluorescein angiograms because of the masking effect of fluorescein which diffuses from the choriocapillaris into the intervascular space (Flower et al 1977; Hyvärinen & Maumenee 1971). Figs 5A and 5B show an area of delayed filling in the choroid but in corresponding fluorescein angiograms (Figs 5C and 5D) no such area of delayed filling is detectable.

sofar ICG angiography has not been used for clinical diagnosis but the findings occasionally affected clinical decisions. In Fig 6A a large submacular neovascularization with a large feeding artery is shown. The draining vein could not be seen in the fluorescein angiograms clearly enough to warrant photocoagulation of the feeder at the disc margin. In the ICG angiogram (Fig 6B) the vein can be seen to follow the artery only half way to the disc. Based upon this observation photocoagulation was made at the disc margin and the neovascularization collapsed without danger of venous bleeding.

Discussion

Development of ICG angiography has been ongoing for several years. The method which used adaptors and an otherwise unmodified Zeiss fundus camera given some insight into the choroidal circulation (Graandijk & van Breijl-Forsius et al 1977). The technical refinements now make possible laser choroidal angiography in clinical investigations. Before it can be used as a diagnostic tool however a fairly large number of studies are needed to derive basic interpretations of different circulatory patterns. At the present time ICG angiography has already demonstrated that fluorescein angiography can give a false impression of circulatory status in some clinical conditions.

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ELECTRORETINOGRAM (ERG) AND VISUAL EVOKED RESPONSE (VER) STUDIES IN PATIENTS WITH OPTIC DISC DRUSEN

BY

EILA MUSTONEN, ILMAR SULG and TAPANI KALLANRANTA

Non-corneal ERGs recorded from infraorbital skin electrodes to flash stimulation and mid-occipital and parasagittal VERs to both flash stimulation and pattern reversal were performed in 26 patients with optic disc drusen. ERGs were normal in all patients. The mean VER amplitude was lower in the eyes with optic disc drusen than the mean amplitude of VERs in the normals but the interindividual variation was also so great in normals that the difference was not significant. The waveform of the major positive peak was quite often broad or split. VER latencies were usually in normal range although the visual field defects could be rather severe. Some other cause was present when the major positive peak was delayed.

Key words: drusen, ERG - half field stimulation - hyaline bodies, optic disc - perimetry - skin electrodes, VER.

Optic disc drusen are anomalous homogeneous often calcified hyaline bodies of the optic nerve heads situated in front of the lamina cribrosa. They can bring about visual field defects presumably by compression causing atrophy of the adjacent nerve fibres or on a vascular ischaemic basis. Visual fields may reveal enlargement of the blind spot, nerve fibre bundle arcuate scotomas, nasal sector or quadrant defects or peculiar irregular concentric contractions (Lorentzen 1966, Savino et al 1979). Central visual acuity usually remains normal (Lansche & Rucker 1957, Rucker & Hearn 1961, Pietruschka & Priess 1973, Erkkila 1977, Foltzsch et al 1978, Rosenberg et al 1979).

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VER has been found to be very sensitive for detecting demyelinated optic nerves in multiple sclerosis (Halliday et al 1972 1973 Miller & Asselman et al 1975 Regan et al 1975 Wildberger 1976 Lowenstein & Bynke et al 1977 Chalm et al 1977 Celesta & Daly 1977 Massouh & Zeese 1977 Hennerici et al 1977 Hoepfner 1978 Shahrokh et al 1978) when visual acuity visual fields and optic discs are normal and there is no retrobulbar neuritis or papillitis. Increase in the latency of the potential of the occipital cortex by pattern stimulation of the retina is the typical finding. It has also been demonstrated by flash stimulation (Richey et al 1971 Miller & Enns 1972 Feinsod & Hoyt 1975 Paty et al 1976 Ellenberger & Zegen 1976) in multiple sclerosis.

VER delays have been found also in spinocerebellar degeneration and atrophy in dominantly inherited isolated optic atrophy and in compression of the optic nerve (Halliday et al 1973 Asselman et al 1973) as well as in optic neuropathy (Hennerici et al 1977) and in Leber hereditary optic atrophy (Nikoskelainen et al 1977). Bornstein (1973) observed delayed responses in atrophy trauma compression and vascular disease. Delayed pattern responses have also been reported in glaucoma (Cappin & Nissim 1975 Bartl 1976).

Compressive lesions of the anterior visual pathways have been found to cause abnormal pattern responses (Halliday et al 1976) even when other clinical signs of visual impairment were absent and visual acuity visual fields and fundus were unaffected but the character of the changes was different from that seen in primary demyelinating disease. The incidence of delayed responses was much lower and the magnitude of the delays smaller than with multiple sclerosis. Absent responses and abnormalities of the waveform of the responses were also reported.

We have not found in the literature reports of simultaneous ERC and VER studies in patients with optic disc drusen except one patient mentioned by Bartl (1977) and a short comment by Neetens & Burvenich (1977) upon the diagnosis of optic disc drusen. As a part of more comprehensive examination of patients with optic disc drusen non-corneal ERCs to flash stimulation and mid occipital and parasagittal VERs both to flash stimulation and pattern reversal were performed in 26 patients and the results are presented in this report.

Material and Methods

In 26 patients (4 with unilateral and 22 with bilateral optic disc drusen) both non-corneal ERCs to flash stimulation and mid occipital and parasagittal VERs to flash stimulation and pattern reversal were performed. Seventeen patients with optic disc drusen were also examined by half field stimulation method (Barrett et al 1971) by using non-occipital electrodes.

mod was the same as in our report of skin electrodes in ERG (Mustonen & Sulg 1980).
 After adaptation in weak room illumination (< 3 lux) for 90 min followed by 30 min in
 as the patient was stimulated with 10 flashes (1000 lux) of 10 msec duration and
 of 0.2 joule from an Elema-Siemens photostimulator (EMT 92) with a lighted area
 ≈ 30 mm giving white light flashes with 10 second intervals 90 cm in front of the
 eyes. Each eye was stimulated separately, the other eye carefully covered by an eye
 the adequacy of the cover was verified by the absence of an ERG from the covered eye.
 A triggered ERG was then recorded with stimulation by 30 flashes at 1 Hz frequency.
 Simultaneous sagittal VER was recorded. Reverse pattern stimulator (Dgtrimer II 110)
 used for subsequent VER examinations. The patient sat facing a translucent screen,
 60 cm at the eye onto which the slide of a black and white checkerboard was
 projected. The individual squares subtended 60 at the eye. The recordings were made
 during each eye separately, the patient being asked to fixate a small red spot in the centre
 screen while the responses were being averaged. Pattern reversal was produced once
 second by a rapid displacement of the checkerboard through one square. The overall
 angle of the field did not change. The lights were off in the examination room but the
 red light kept the room weakly illuminated. The average response to 30 reversals was
 recorded. In 17 patients also half field stimulation was used. The averaging time was 900
 ms.

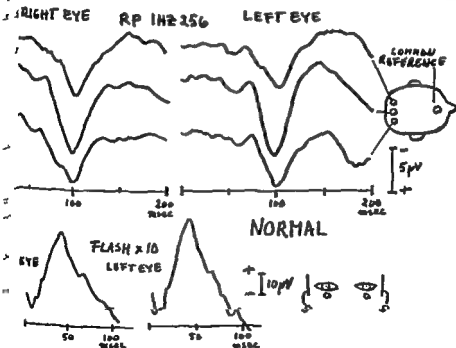


Fig 1

Corneal ERGs with stimulation by 10 flashes at 10-second intervals and mid-occipital and
 sagittal VERs with stimulation by 30 pattern reversals at 1 Hz frequency in one normal
 subject.

ERGs were recorded from infraorbital skin electrodes with the ipsilateral eye as reference and the usual EEG electrodes at the vertex as the ground ones. Clear disc electrodes 10 mm in diameter were attached by an adhesive ring to the lower eyelid at the centre of the pupil close to the lid margin and the contact was confirmed by a paste. VERs were recorded from three occipital electrodes all referred to a electrode 12 cm above the nasion. The mid-occipital electrode for visual VER was 7 cm above theinion and parasagittal VERs were recorded from electrodes 5 cm to the left from the mid-occipital one.

The primary amplification and recording of both ERG and VER was performed on a 16-channel Elema Siemens EEG-machine (Mingograph Universal) time constant 0.1 s, high frequency cut 70 Hz. From the Mingograph the amplified signals were transferred to a 2-channel 1000 points signal analyser averager (Hewlett Packard 5401). The responses were documented by means of Polaroid photographs from the CRT of the oscilloscope of the signal averager (Fig. 1).

Table 1

ERG. The latencies (measured from the stimulus-on) and the amplitudes of the b-wave (measured from the trough of a waves) recorded from lower lid skin electrodes in 49 subjects (49 eyes) and in 26 patients with optic disc drusen (48 eyes) with stimulation by 15 flashes at 15 second intervals. Same parameters in 15 of normal subjects (15 eyes) and in 26 patients with optic disc drusen (48 eyes) with stimulation by 32 flashes at 0.2 Hz.

	Normal						Optic disc drusen					
	Latency (msec)			Amplitude (μ V)			Latency (msec)			Amplitude (μ V)		
	b-wave			b-wave			b-wave			b-wave		
	10 flashes at 15 second intervals (0.06 Hz)											
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Right eye	39.8 \pm 2.76	(22)	31.5 \pm 13.22	(92)	39.7 \pm 4.69	(3)	40.9					
Left eye	39.6 \pm 3.21	(21)	33.7 \pm 13.40	(21)	39.4 \pm 4.26	(5)	35.5					
Average R&L eye	39.7 \pm 2.99	(43)	32.6 \pm 13.31	(43)	39.4 \pm 4.44	(4)	38.2					
32 flashes at 0.2 Hz frequency (0.2 Hz)												
Right eye	39.6 \pm 2.71	(15)	24.0 \pm 11.89	(15)	40.3 \pm 3.60	(5)	31.1					
Left eye	39.8 \pm 2.19	(14)	25.8 \pm 14.67	(14)	40.0 \pm 4.10	(5)	24.5					
Average R&L eye	39.7 \pm 2.45	(29)	24.9 \pm 13.99	(29)	40.1 \pm 3.85	(10)	27.8					

Results

patients with optic disc drusen showed from both eyes non-corneal ERGs normal latency and normal amplitude of the b-wave the latency measured from the stimulus-on and the amplitude from the trough of the a wave (Table I) the latency and amplitude of the a wave were also in normal range

the peak latency of the major positive wave of pattern VER from the midline and parasagittal electrodes and the peak to-peak amplitude of the major positive wave measured to the immediately following negative peak were determined

The results were compared with those from 21 normal subjects. The amplitude of the major positive peak was more variable than the latency also in normal subjects. The upper limit of normal for the latency of the peak of the major positive potential in the pattern VER was considered to be the mean + 3 SDs which was about 111 mseconds

Table II

The latencies (measured from the pattern reversal) and the amplitudes of the major positive component of VER (measured to the immediately following negative peak) recorded from occipital electrodes in 21 normal subjects (41 eyes) and in 24 patients with optic disc drusen (42 eyes) with stimulation by 2.6 pattern reversals at 1 Hz frequency. Some results from parasagittal electrodes are also presented. The fellow-eye in unilateral drusen was not included in the numbers

	Normal						Optic disc drusen					
	Largest positive wave						Largest positive wave					
	2.6 pattern reversals at 1 Hz frequency											
	Latency (msec)			Amplitude (μ V)			Latency (msec)			Amplitude (μ V)		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
<i>occipital electrode</i>												
eye	99.3	4.20	(21)	10.8	± 5.4	(21)	98.7	± 9.98	(20)	6.8	± 3.84	(20)
eye	98.6	± 3.76	(20)	11.1	± 5.86	(20)	100.1	± 15.71	(22)	5.4	± 3.34	(22)
Left eye	99.0	± 3.98	(41)	11.0	± 5.70	(41)	99.4	± 12.85	(42)	6.1	± 3.59	(41)
<i>sagittal electrode</i>												
eye to contralateral eye	99.6	± 6.35	(37)	6.7	± 3.69	(34)	98.5	± 13.98	(40)	5.0	± 2.39	(41)
eye to ipsilateral eye	100.2	± 5.69	(33)	7.5	± 4.01	(30)	98.9	± 10.30	(43)	5.3	± 3.65	(43)

The whole group of 26 patients with optic disc drusen was so heterogeneous that the direct comparing of their VERs with those in normal subjects (Table 1) reveals that the mean latency was the same as in normals but with larger standard deviation and that the mean amplitude was lower than in normals. This difference was not significant because of the great interindividual variation in amplitudes also in normal subjects. The amplitude of the major positive peak varied from 3.1 μ V to 26.2 μ V in normals but under 5 μ V it was only in three cases (3 eyes) whereas the variation in patients with optic disc drusen was from 1.5 to 15.5 μ V and in 13 patients (20 eyes) the amplitude was under 3 μ V. The shape of the response was altered in eight cases with optic disc drusen.

Seven different subgroups of patients with optic disc drusen are separately examined. Visual fields were registered with both Goldmann and Friedmann methods.

1 Two patients had unilateral optic disc drusen with normal visual acuity but with a slight visual field defect with an enlarged blind spot in the left eye with optic disc drusen. The latencies of the major positive peak were normal but amplitudes were low in both eyes (3.6 μ V and 3.1 μ V, 3.6 μ V and 2.7 μ V in the right and left eye respectively).

2 Two patients had unilateral optic disc drusen with decreased visual acuity and a field defect in that eye. The first patient with drusen in her right optic disc had chorioretinal scars near the macula causing decreased vision and small paracentral defects in the visual field. VER amplitudes were low in both eyes (4.3 μ V and 4.6 μ V in the right and left eye respectively) and the latencies were normal with no difference between the eyes. The second patient with drusen in his left optic disc had ten years previously experienced a sudden decrease of vision with a temporal field cut in that eye. Afterwards an inferior defect also developed and the optic disc became pale. VER latency was normal (normal range) in that eye and amplitude was lower than in the right eye (9.8 μ V and 3.1 μ V). A measurable VER was got with left half field stimulation of that eye. The other eye gave normal results also with half field stimulation.

3 Three of the patients with bilateral optic disc drusen had normal visual acuity and normal visual fields in both eyes. VERs showed normal latencies, amplitudes and shapes.

4 Five patients with bilateral optic disc drusen and normal visual acuity in both eyes showed unilateral minor visual field defects. However, no significant difference between the right and the left eye could be found in VER curves. Two patients revealed bilateral major positive waves and low amplitudes in both eyes; the VERs of the other three were also to half field stimulation.

5 Ten patients had bilateral optic disc drusen with normal visual acuity but with bilateral field defects in both eyes. Amplitudes could be low although the field defects were not severe. In four patients the amplitudes were under 3 μ V in both eyes. Latencies of the major positive wave were in normal range in all eyes and no significant difference between the eyes could be found although the visual field defects could be rather different in the eyes (Fig. 2). The waveform of the major positive wave was broad in many cases and with no distinct peaks. Half field stimulation used in seven patients gave better results with the ipsilateral parasagittal electrode as it did in normals without any correlation with the visual field defect that left the most central field intact.

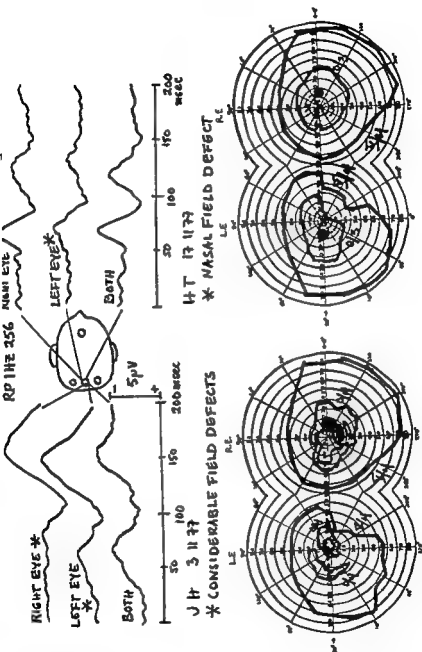


Fig -
 Topocal VERs with stimulation by 9 II pattern reversals at 1 Hz frequency in two patients with bilateral optic disc drusen and visual field defects

6 In two patients with bilateral optic disc drusen the visual acuity was decreased in one eye. One had amblyopia ex anopsia from uncorrected hyperopia and astigmatism but also a considerable nasal field defect. The latencies of the major positive peaks were longer in both eyes than in normals, more so in the left amblyopic eye (111 mseconds in the right and 124 mseconds in the left eye). The other patient had a considerable visual field defect in the eye with decreased vision and also a peripheral defect in the other eye but VEPs were in normal range in both eyes.

7 The last small subgroup included two patients with bilateral optic disc drusen and bilaterally decreased visual acuity. One patient was examined four years after operation for radiation of pituitary adenoma. Before operation he had bitemporal field defects which improved after therapy (Mustonen 1977). Major positive peaks were delayed (129 mseconds in the right and 130 mseconds in the left eye) with some moderate asymmetry from parasagittal electrodes typical for bitemporal hemianopia and optic nerve compression (Blumhardt et al. 1977). The other patient with bilateral optic disc drusen and decreased vision in both eyes showed delayed major positive peaks (135 mseconds in the right and 121 mseconds in the left eye) and amplitudes were low. He experienced difficulties with colour vision since childhood when he could not distinguish blueberries or yellow cloudberries but could well discern red whortleberries from green leaves. Now he showed grave colour vision defects and visual fields revealed bilateral superior and inferior scotomas. Optic discs were rather pale with nasally situated drusen, and arterioles were somewhat accentuated. Foveal reflexes were absent and maculae were dark and atrophic. Dark adaptation revealed a cone plateau of very short duration but final adaptation was normal as well as ERGs after dark adaptation. Fluorescein angiography showed late-phase leakage in the area of pigment epithelium disturbance at both maculae. He also had a family history of progressive cone dystrophy. Family history revealed no other similar case.

Discussion

Straub (1961) reported normal ERGs in patients with optic disc drusen. Subnormal ERGs were found by Elenius et al. (1961) in a case of tapetoretinal degeneration combined with drusen of the optic discs and glaucoma simplex. Vajnter et al. (1958) reported subnormal or normal ERGs in three slightly night-blind patients with optic disc drusen. In the same family these authors also found abnormal ERGs in two other siblings with fundus changes typical of retinitis punctata serena. In a case of optic disc drusen Ayesb et al. (1976) presented a case with optic disc drusen and retinitis pigmentosa and retinal telangiectasia (Coats's disease). Both the scotopic and photopic ERGs were almost totally extinguished. In our 76 patients with optic disc drusen non-corneal ERGs were normal but the material did not include three patients with retinitis pigmentosa and two patients with late-onset juvenile retinoschisis and optic disc drusen. Babel et al. (1971) in their book on Electrophysiology described one patient with optic disc drusen whose ERG was normal and VEPs of normal latency but with a split major positive wave.

Jurvenich (1977) mentioned absolutely normal flash VER and PVER in a patient with optic disc drusen and a slight enlargement of the blind spot. Our series of patients with optic disc drusen showed that in half of the cases VER was absolutely normal and in another half the amplitudes were rather low resulting in a lower mean amplitude than in normal subjects but the interindividual variation is great also in normals that the difference was not significant. The waveform of the major positive peak was quite often broad or split (eight cases of all) and the latencies were usually in normal range although the visual field defects could be severe. Therefore some other cause should be thought if the latency of the first positive peak in pattern VER is delayed in patients with optic disc drusen.

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	Age at follow up	Grade of vitreous staining	Cornea slit lamp	Pachymetry			Endothelial cell density	Visual function		
				Before operation	Latent stained	Central postoperative last examination stained		Visual stained eye	Central stained eye last examination	
1	67 M	1	opacity	0.48	0.48	0.45	1979	1612	ff	<ff
2	68 M	2	opacity	0.54	0.51	0.50	1992	1860	ff	66
3	69 M	1	opacity	0.57	0.49	0.47	2007	2135	bb	ff 36
4	70 F	3	opacity	0.50	0.50	0.51	219	2190	ff	ff
5	72 F	2	opacity	0.3	0.53	0.6	1538	1240	<ff	<ff
6	70 F	0	opacity	0.53	0.50	0.5	034	2147	ff	ff
7	76 F	3	opacity	0.51	0.58	0.48	999	1637	<ff	ff 18
8	78 M	2	opacity	0.47	0.45	0.45	2383	2418	ff	<ff
9	78 F	0	opacity	0.51	0.54	0.56	1841	2001	>ff 12	69
10	79 M	3	opacity	0.48	0.51	0.50	1649	1849	ff	1**
11	80 M	1	opacity	0.69	0.59	0.5	190	184	<ff 12	ff
12	82 F	1	opacity	0.45	0.44	0.49	1959	1498	ff 24	ff
13	8 F	2	opacity	0.49	0.5	0.53	137	193	ff 12	ff
14	90 F	0	opacity	0.51	0.50	0.48	2377	2117	160	160
15	9 F	2	opacity	0.53	0.43	0.49	2336	2117	ff	<ff 10
mean				0.59	0.50	0.51	1839	1844		
± SEM				±0.01	±0.03	±0.01	±116	±120		

* Preoperatively operated on for glaucoma; ** Postoperatively

Table II

Aphakic eye vital stained by trypan blue compared with contralateral non-operated control eye. Eight patients examined by scleral scatter pachymetry and endothelial cell count

	Age at follow up	Crude of vital staining	Corneal slough	Pachymetry			Endothelial cell density		Vision
				Before operation	Last exam vital stained	Contralateral exam vision at time	Vital stained eye	Contralateral exam vision at time	
1	631	4	opacity	0.48	0.78	0.43	—	—	1/36*
2	131	3	opacity	0.1	0.30	0.53	1,218	2,500	6/6
3	131	—	opacity	0.30	0.22	0.53	2,911	932	6/6
4	7131	1	opacity	0.12	0.4	0.6	1,138	—	1/36
5	821	3	opacity	0.20	0.17	0.13	2,111	—	1/24*
6	811	2	opacity	0.9	0.3	0.1	1,644	2,088	2/40
7	871	1	opacity	0.17	0.1	0.7	2,028	—	1/3
Mean (s.d.)				0.28	0.46	0.49	116	2,028	1/1

Table III

man aged 61 Vital staining by trypan blue during cataract extraction on a pronounced senile macular degeneration of the retina and sequels of acute glaucoma in both eyes

Grade of vital staining	Cornea slit lamp	Pachymetry		Endothelial cell density	Vision
		initially	last exam		
1	nat	0.46	0.52	1706	1/60
3	nat	0.59	0.50	1579	H + P

Material

operative vital staining was performed on 47 patients subjected to cataract extraction (Norm 1971). These were followed up 6 to 12 months later (Norm 1973). At the time of the present investigation eight years after the operation 22 had died, one had emigrated (address unknown). The remaining 24 (51% of the series) had been summoned and all appeared for examination. Age at follow up and sex are shown in Tables I, II and III.

Seventeen had both eyes operated on for cataract. The one eye had been vital stained while the contralateral eye acted as an aphakic control eye (Table I). Eight had only one eye operated on and vital stained during operation (Table III).

Methods

Trypan blue was an aqueous solution for intravenous use having no salts added, isotonicity nor buffer.

The procedure of cataract extraction has been described previously (Norm 1971). Ha-chymotrypsin was employed for all aged under 60, a total of five trypanblue stained eyes.

In the eight year follow up the cornea was examined in the slit lamp, microscopically for cloudiness. Using scleral scatter technique the eyes were examined for corneal oedema (Norm 1974).

The central thickness of the cornea was measured by means of Haag Streit's pachymeter and Kaufmann's specular endothelium microscope (Knight et al 1978).

The number of endothelial cells centrally on the cornea was calculated on the basis of the best three photographs out of about nine takings per eye using

Kaufmann's contact endothelium microscope. Sperling's method was used for the counting to avoid counting each cell more than once (Sperling et al.)

Results

The results are shown in Tables I–III. The cornea showed no oedema on examination. Corneal opacity was observed in only four of the visualized six of the control eyes. These corneal opacities were seen in patients with open glaucoma (two + two control eyes), band shaped calcareous keratopathy (one control eye), metaherpetic keratopathy (one control eye), phthisis (one eye) and opacity over the area of the operation scar (one + one control eye).

A visual acuity of 6/12 or better ($\geq 20/40$) was obtained in 13 out of 23 aphakic eyes. The visual impairment of the remaining 12 eyes was due to macular degeneration ($n = 8$) or – though rarely – keratopathy before (three) or strabismic amblyopia (one).

The corneal thickness averaged 0.52 mm prior to the cataract extraction. In control eight years later the thickness was unchanged as assessed by the Haag Streit pachymeter. It did not differ significantly from the operated eye (Table I) nor from the non-operated eye (Table II).

Neither did specular microscopy reveal any significant differences.

There was no difference in central corneal endothelial cell density between the vital stained eye and the corresponding aphakic control eye (Table II). The cell density was of course reduced after cataract extraction (Table II).

The coefficient of variability of the first two measurable duplicate measurements was 12% ($n = 36$ out of 42).

The grade of trypan blue vital staining of the endothelium preoperatively and the use of alpha-chymotrypsin do not seem to influence the parameters studied.

Panophthalmia or marked iris were not seen in the observation period.

Comments

Vital staining seemed not to be the cause of endothelial cell damage as there was no difference in cell counting and pachymetry.

The endothelial cell count was found to have a high coefficient of variability, presumably because a number of the old patients were unable to cooperate sufficiently. The above mentioned conclusion must therefore be taken with reservation.

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PAS POSITIVE POLYMORPHONUCLEAR LEUCOCYTES IN CORNEAL ULCERS

BY

J U PRAUSE and O A JENSEN

Fifty consecutive cases of severe keratitis with clinical signs of corneal ulcer were examined histologically for the stage of inflammatory reaction. In 14 similar cases were studied ultrastructurally.

Cell counts of polymorphonuclears (PMN), eosinophils, lymphocytes and plasma cells were carried out. Further the corneas were grouped according to their content of PAS positive PMN. The state of ulceration was assessed microscopically.

PMN at a cell count of > 10 cells/mean field ($\times 1000$) were found in 26 cases and were significantly more frequent in corneal areas with epithelial and Descemet membrane defects. Most PMN were PAS-positive ($> 70\%$) positive material having the characteristics of glycogen. PAS-negative PMN were found in corneas with increased amount of plasma cells.

By TEM the PMN were found highly phagocytic, partly degranulated and reduced glycogen content. TEM could not on the formalin fixed specimens determine whether PAS negative PMN had a higher protein turnover. Our findings indicate that corneal ulcers with PAS-negative PMN contain a high amount of proteases because of many degranulated PMN and probably reactivation of PMN. Therefore the PAS stainability can be used as an indicator for the PMN activity in corneal ulcers.

Key words: corneal ulcer - polymorphonuclear leucocytes - eosinophils - granulocytes - lymphocytes - plasma cells - PAS-staining - tissue turnover.

as been published concerning the stainability of the neutrophilic polymorph leucocytes (PML) in histological sections of inflamed corneas. As the of PAS-positive PML in corneal lesions was observed to vary, the aim of the study was to determine the material which was responsible for the PAS reaction and to reveal any relationship between the presence in the cornea of positive PML and the severity of the corneal lesion.

Material and Methods

The microscopic part of the study comprised 50 consecutive cases of severe keratitis. Clinical diagnosis of corneal ulcers referred to the Eye Pathology Institute (1970–74). The material consisted of 28 males and 22 females. The median age of patients was 74 years (range 3–99 years). The preceding history of corneal disease had a median duration of 3 years (range 3 months–approx. 300 years). The aetiology of the cases is stated in Table I.

The tissues had been fixed in 10% buffered neutral formalin for 24 h, processed according to the paraffin technique and cut in 8 μ m sections.

Raffinized, hydrated sections were digested for 1 h at 37°C with 0.5% hog pancreas α -amylase (type VI A M lot 76C 0096, Sigma, St. Louis) in 0.5 M phosphate buffer, pH 6.5. The enzyme concentration used in this study was determined in a pilot study with a dilution of α -amylase (0.5–0.05%). Neighbouring control sections were treated with phosphate buffered saline.

Sections were stained with periodic acid–Schiff (PAS) or McManus (PAS). Some PAS-stained sections were digested and re-stained.

In every five sections from each patient were examined by light microscopy (LM) ($\times 400$). Defects in the corneal layers recorded. Defects in the epithelium were only counted when sections showed a total loss of epithelium in the ulcer area. Defects in the endothelium were recorded as they were often due to trauma caused by the technical procedure.

Table I
Aetiology of corneal ulcers

Aetiology	Number
Posttraumatic	4
Following cataract extraction	6
Graft rejection	4
Exposure keratitis	5
Bacterial infection	5
Viral infections (Herpes simplex)	10
Other	16
Total	50

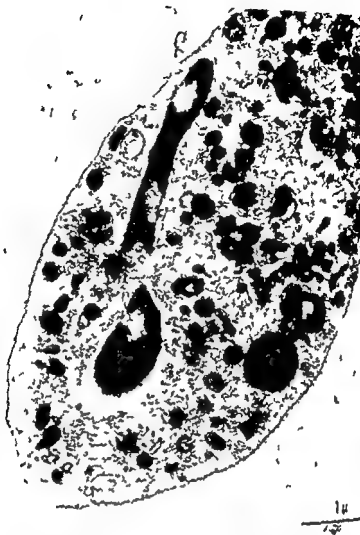


Fig 2

Electron micrograph of PAS-positive PML showing dispersed glycogen (C) and several phagosomes (P) (Lab No J67/18 TEM 309 × 45)

PML were significantly more frequent ($P < 0.05$) in sections with rupture or defects in Descemet's membrane. PAS negative PML were found in plasma cells but there were no differences between the groups concerning relation to the other examined parameters.

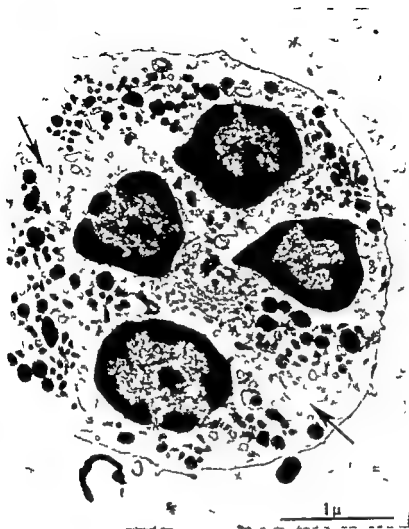


Fig. 4

digested specimen showing PML with loss of cytoplasmic glycogen leaving clear cytoplasmic areas (arrows) (Lab No 992 78 TFM 346 \times 28 500)

Examination The PML were naturally not so well preserved as in tissue properly processed for TEM. However, the nucleus and cytoplasm were quite well seen, the plasma membrane being sometimes destroyed. The PAS-positive glycogen in the cytoplasm abundant dispersed β -particles of glycogen digestible



Fig 3

PML in liquefied part of ulcer with only a few granules sparse of phagosomes (P) containing material of same morphology as the surrounding collagen (C) (Lab No "93/79 TEM 436 x 10 000)

by α amylase (Figs 3-4). In addition these PML showed several phagosomes of various sizes. They contained a granular material of the same structure as degraded collagen surrounding the cells (Figs 3-5). In less destroyed corneal stroma macrophages with phagocytosed PML were found (Fig 6).

Discussion

Wu et al (1979) found granulocytes containing PAS-positive material but this was amylase resistant in contrast to our PAS-positive material. The usual histological paraffin technique has been considered as removing glycogen. This however is not the case (Pargle 1963 Poulsen 1979). Glycogen is not digested by amylases and it is therefore very probable that the α amylase sensitive material observed in the PML was glycogen. Furthermore pilot studies using different concentrations of α amylase ensured that the enzyme concentration employed was sufficient to exclude any false amylase reaction.

By TEM the presence of glycogen in PAS-positive PML was established. The glycogen was seen as β -particles digestable by amylase.

Prometamyelocytes have only little glycogen (Staples & Getaz 1977) and are non-azurophilic granules containing most of the tissue degrading enzymes such as hyaluronidase, cathepsin G and collagenolytic serine protease (Rowsey et al 1973; Heiser et al 1976; Starkey & Barret 1976; Ohlsson et al 1977).

Glycogen particles accumulate in metamyelocytes and increase with maturation. An increase in glycogen content is followed by an increase in number of specific granules (Bainton et al 1971) containing collagenase (Baggiolini 1979). At the same time the number of azurophilic granules is reduced.

Little glycogen is found in the mature circulating PML, which contain a limited number of granules. These PML represent the non-dividing non-secretory end stage of the cells (Staples & Getaz 1977). Since the PAS-positive PML are end stage cells with limited protease content, the tissue destructive capacity in a corneal ulcer is related to the number of PML present, and if a replenish with PML is prevented, as elegantly shown by Kenyon et al (1979) the destructive capacity therefore is reduced. Our findings confirm, on a human material, the experimental findings of Kenyon et al (1979) especially the presence of the phagocytic active partly degranulated PML with a variable content of glycogen found in the melting areas of the ulcer. We also found macrophages containing PML remnants in the more peripheral parts of the ulcer (Fig. 6).

The PAS-negative PML were found together with plasma cells and by TEM they were often found in melting areas of the cornea.

The PAS-negative PML can contain nearly phagocytosable material, but they are not active. Unfortunately, our routine formalin fixed specimens do not allow a definitive TEM examination of the Golgi apparatus and endoplasmic reticulum. It is therefore impossible to decide whether the PAS-negative PML are former PAS-positive PML which have degranulated and at the same time have used up their glycogen content and are now dying. However, it could also be PML which are activated to a prometamyelocytic stage rebuilding their granules i.e. reactivated.

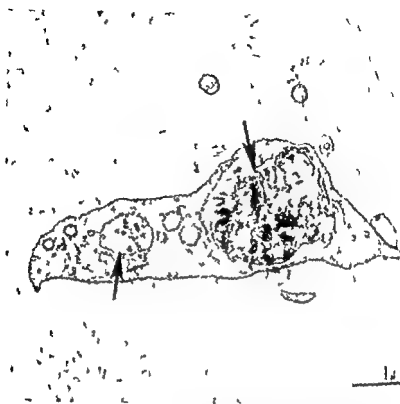


Fig 6

Area in non liquefied part of ulcerated cornea with macrophage having phagosomes (arrows) The cell is surrounded by regularly arranged collagen fibres (Lab. TEM 979 \times 74 000)

like lymphocytes. The PAS negative PML are not genuine promeak cells as they contain mature lobulated nuclei.

The presence of plasma cells together with PAS-negative PML are of interest because when PML is phagocytosing immune complexes enzymes are released (Mowat 1979).

Thus a corneal ulcer with a high amount of PAS-negative PML shows increased liberation of tissue destroying proteases as found in human corneal ulcers.

Our findings may therefore support PML proteases responsible for the stromal liquefaction found in immunologically active corneal ulcers (Sjolle 1978) and the high collagenolytic activity found in corneal tissue under PML (Piric et al. 1977).

The present study was not designed to take account of the mechanical

before enucleation — and the use of various antibiotics or particularly of corticosteroids factors which might be of importance to our findings. This study also confirms earlier findings that PML mainly accumulate in denuded part of the cornea (Haik & Jimmy 1977 Sheppard et al 1971) which indicates that part of the PML are brought to the ulcer by the tear fluid (Wine & Basu 1958 & Kuwabara 1962).

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BACTERIAL FLORA IN RELATION TO CATARACT EXTRACTION

V Effects of Topical Antibiotics on the Preoperative Conjunctival Flora

BY

J. A. FAHMY

In a randomized study comprising 60 patients six different prophylactic regimens were evaluated for their effectiveness in eradicating bacteria on the conjunctivas before surgery. Gentamicin sulfate ophthalmic solution was the only antibiotic able to eliminate bacteria in almost all of the examined patients. The other drugs (chloramphenicol solution oxytetracycline polymyxin B ointment, sulphamethizole bacitracin neomycin and ristocetin polymyxin B solutions) were not effective although most of the isolated strains were sensitive to the respective antibiotics probably because of the short treatment period (18 h). The problem of prophylactic therapy in ophthalmology is discussed and some guidelines are given.

Am uo ds bacteria - conjunctiva - antibiotics - cataract extraction - endophthalmitis - prophylaxis

prophylactic role of topical antibiotics administered prior to intraocular surgery is still the subject of dispute. Several authors (Dunnington & Locatcher 1940 Khorazo 1943 Callahan 1953 Locatcher 1953 Locatcher Khorazo & Gutierrez 1956 1972 Allen & Mangione 1964 1974) stated that the elimination reduction or suppression of conjunctival ocular flora - the principal remaining hazard in intraocular surgery - significantly reduce the occurrence of postoperative infection. Others (Leopold 1958 Leopold & Apt 1960 Goodner 1963 Leopold 1971 1972) disagreed and claimed that the surgical field in ophthalmology cannot be completely sterilized and the use of topical antibiotics may lead to the alteration of the local

Table I
Age and sex distribution

Age	Sex		Total
	F	M	
≤ 50	2		2
51-60	3	2	5
61-70	14	8	22
71-80	11	4	15
≥ 80	3	11	14
Total	33	23	60

conjunctival flora permitting the development of pathogens which proliferate because competitive organisms have been inhibited. Further the use of lactic therapy may allow one to develop or encourage the multiplication of strains.

Although the subject is of much interest only insufficient data exist at the time to determine the effect of short time usage of antibiotics on the flora. It has therefore been the main purpose of the present study to solve this problem.

Material and Methods

The material comprises 60 patients admitted to the Department of Ophthalmology, Kommunehospitalet, Copenhagen during the period 14.11.1977 for cataract extraction. The age and sex distribution can be seen from Table I.

Only cases without any signs of inflammation or infection were included. Further it was made certain that none of them had received any systemic corticosteroids during the last months prior to operation.

The patients received randomly one of the following drugs: 1) chloramphenicol ophthalmic solution 0.5%, 2) gentamicin sulphate ophthalmic solution 0.3%, 3) oxytetracycline chloride 3% - polymyxin B sulphate 0.1% ophthalmic solution, 4) sulphamethizole ophthalmic solution 4%, 5) bacitracin 1% - neomycin 0.3% ophthalmic solution and 6) ristocetin sulphate 0.2% - polymyxin B sulphate 0.2% ophthalmic solution. Thus each treatment group included 10 patients. Each drug was applied 5 times starting shortly after admission (approximately 1 hour before surgery) and ending at bed time.

enial cultures were taken before the antibiotic treatment, and again the next morning before operation. Methods of obtaining cultures, identification of strains and preparation for surgery were similar to those described elsewhere (et al. 1970a, b).

Results

Chloramphenicol

Table II it may be seen that a total of 22 bacterial strains were isolated before treatment. Chloramphenicol was effective in the elimination of 10 strains while 12 strains remained uninfluenced. Six new strains were recovered after treatment and the sign test was applied this effect was found not significant ($P > 0.05$). Sensitivity tests were performed and showed that six strains (all belonged to *Stylococcus albus*) out of 40 were resistant to chloramphenicol (22 isolates before and 18 after treatment).

Gentamicin

Effective in the elimination of 21 out of 22 isolated strains (Table III). One *Stylococcus albus* remained uninfluenced while no new strains were recovered after treatment (sign test $P < 0.01$). None of the strains were resistant to gentamicin (total number of isolates is 23).

Table II

Effect of chloramphenicol eye drops on bacterial strains isolated from 10 normal non infected eyes

Microorganisms	Treatment	Number of strains			
	Before	+ growth	+ growth	- growth	- growth
	After	+ growth	- growth	+ growth	- growth
<i>Stylococcus albus</i>		9	4	5	
<i>Stylobacillus</i>			2		
<i>Staphylococcus aureus</i>		9			
<i>Streptococcus non haemolyticus</i>		1	9	1	
<i>Enterobacter aerogenes</i>			1		
Unidentified gram negative bacilli			1		

Table III

Effect of gentamicin eye drops on bacterial strains isolated from 10 normal eyes

Microorganisms	Treatment	Number of strains			
	Before	+ growth	+ growth	growth	r
	After	+ growth	- growth	+ growth	r
<i>S. albus</i>		1		11	
<i>Corynebacteria</i>				2	
<i>S. aureus</i>				1	
<i>Streptococcus non</i> <i>haemolyticus</i>				9	
<i>faecalis</i>				1	
<i>B. anthracis</i>				3	
Unidentified gram negative bacilli				1	

Oxytetracycline - polymyxin B

was ineffective against 13 out of 17 strains two new strains were recovered after treatment (sign test $P > 0.05$) (Table IV). Four strains *S. albus* were resistant to oxytetracycline two *S. aureus* to polymyxin B and one strain *Proteus mirabilis* to both antibiotics (total number of isolates is 30).

Table IV

Effect of oxytetracycline polymyxin eye ointment on bacterial strains isolated from 10 non infected eyes

Microorganisms	Treatment	Number of strains			
	Before	+ growth	+ growth	- growth	r
	After	+ growth	- growth	+ growth	r
<i>S. albus</i>		9	2	1	
<i>Corynebacteria</i>			2		
<i>S. aureus</i>		1		1	
<i>P. mirabilis</i>		1			
<i>E. cloacae</i>			1		
<i>B. anthracis</i>			1		

Table V

of sulphamethizole eye drops on bacterial strains isolated from 10 normal non infected eyes

Microorganisms	Treatment	Number of strains			
	Before	+ growth	+ growth	- growth	- growth
	After	+ growth	- growth	+ growth	- growth
<i>S. albus</i>		10	1	4	
<i>S. neobacteria</i>		1	2		
<i>S. aureus</i>			1		
<i>Streptococcus non haemolyticus</i>			1	1	
<i>S. nitratum</i>				1	

sulphamethizole

ineffective in the elimination of 11 out of 16 strains six new strains were recovered after treatment (Table V) (sign test $P > 0.05$) Only one strain *S. albus* resistant to sulphamethizole (total number of isolates is 33)

fracin-neomycin

ineffective in 12 out of 20 instances four strains were recovered after treatment

Table VI

of fracin-neomycin eye drops on bacterial strains isolated from 10 normal non infected eyes

Microorganisms	Treatment	Number of strains			
	Before	+ growth	+ growth	- growth	- growth
	After	+ growth	- growth	+ growth	- growth
<i>S. albus</i>		10	2	1	
<i>S. neobacteria</i>		1	1	1	
<i>S. aureus</i>		1			
<i>Streptococcus non haemolyticus</i>			3		
<i>S. nitratum</i>			1	1	
unidentified gram negative bacilli			1		

Table VII

Effect of ristocetin polymyxin B eye drops on bacterial strains isolated from infected eyes

Microorganisms	Treatment	Number of strains			
	Before	+ growth	+ growth	growth	—
	After	+ growth	— growth	+ growth	—
<i>S. albus</i>		10	5	1	
<i>Cornebacteria</i>			1	1	
<i>S. aureus</i>		1			
<i>Streptococcus non haemolyticus</i>			2	1	
<i>B. anitratum</i>			1		

(Table VI) (sign test $P > 0.05$) No sensitivity test was performed (total number of isolates is 35)

Ristocetin-polymyxin B

failed to eliminate 11 out of 19 strains three new strains were eliminated (Table VIII) (sign test $P > 0.05$) Three strains (two *S. albus* and *Streptococcus non haemolyticus*) were resistant to polymyxin B and

Table VIII

Effect of topical antibiotics on the conjunctival bacterial flora (total elimination from 60 patients)

Antibiotics	Bacterial growth			
	Before treatment	+	+	—
	After treatment	+	—	—
Chloramphenicol		10	0	0
Gentamicin		1	0	0
Oxytetracycline				1
polymyxin B		10	0	0
Sulphamethizole		10	0	0
Bacitracin neomycin		1	1	0
Ristocetin polymyxin B		1	1	0

The figures indicate number of cases

(one *S. albus* and one *B. anthracis*) were resistant to ristocetin (total number tests 34)

Table VIII shows the effect of the above mentioned drugs on the elimination of bacteria in the respective treatment groups. Gentamicin was able to eradicate all bacteria in nine out of 10 cases, bacitracin, neomycin and ristocetin polymyxin B in one instance each. The other drugs were not able in any case. When these results were compared using the Fisher's exact test, the effect of gentamicin was significantly ($P < 0.002$) superior to the others after pooling their results.

Discussion

In view of the present results, the presence of *S. albus* was definitely considered beside other potential pathogens, since it has been demonstrated that this microorganism may be the cause of postoperative intraocular infection (Linton et al. 1973; Allen & Mangaracine 1974; Forster 1974, 1976; Fahmy

1976). The clinical effectiveness of the examined drugs against infective eye diseases is well established (Leopold & Apt 1960; Norm 1970; Havener 1978). However, their effects on the normal bacterial flora seem to differ. According to the present study, gentamicin was able to suppress the flora totally in almost all of the examined cases, while the other drugs were ineffective, although most of the strains were sensitive to the respective antibiotics and sulphonamide. This may be due to the short treatment period. Locatcher-Khorazo & Gutierrez (1956) found that *S. albus* was usually eliminated in 2-3 days, while gram-negative bacilli persisted as long as 7 days; no information was given as to the time needed to eradicate *S. albus*. The superiority of gentamicin in the treatment of ocular infections (Halasa 1967; Mason & Snie 1967; Gordon 1970), reduction of postoperative flora (Burns et al. 1968, 1972) and pre-operative bacterial counts of the lids (Whitney et al. 1972) has been demonstrated. However, this powerful antibiotic does not seem to be the drug of choice for routine prophylaxis. Its uncritical use will create the resistant strain that may kill tomorrow's patient (Havener 1978). Nonetheless, in certain instances, such as surgery on the last eye, contaminated penetrating trauma or extensive ocular procedures, its application seems to be justified. The fact that most of the examined antibiotics have proved to be ineffective in the elimination of bacteria within the mentioned period of 18 h and the limited effects of gentamicin may stimulate further studies to determine the effects of other antibiotics and eventually other alternatives when used in a longer period of time, e.g. 8-10 days. Such studies, if performed on a larger series of patients, may well have fallen negatively — i.e. if bacteria were not totally eradicated — whether

potential pathogens do flourish and whether resistant strains are claimed by Leopold (1958 1960 1971 1972)

Finally it has to be proved in controlled studies with bacterial cultures the actual value of total eye sterility at the time of surgery and frequency of postoperative infection. Such studies do not exist and are required.

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A HUMAN AND IN VITRO STUDY ON THE EXCHANGE OF AND SOLUTES FROM SOFT CONTACT LENSES

BY

T. SØRENSEN, F. TAAGEHØJ-JENSEN and J. MARGULIES

Elimination of technetium (pertechnetate) in normal saline was studied from various types of contact lenses placed on normal human eyes. Means of computer assisted gamma camera using region of interest technique with the designated area corresponding to the conjunctival sac.

An elimination 4 times slower was found from a highly hydrophilic lens (Scanlens) than from a HEMA lens (Softlens) 0.3% min and 0.1% min respectively. From an ultrathin lens (U3 Softlens) was eliminated 0.4% min. All lenses did not absorb the isotope.

In a laboratory study the lenses were pre-soaked in pertechnetate. Lenses were washed at 2 min intervals in 0.5 ml saline. By this procedure 3% min of technetium was eliminated from Scanlens, 16% min from Softlens and 9% min from a thin therapeutic lens (Plano-T). The ratio Softlens:Scanlens as the human study 1.0 and in the laboratory study 4.9. Radioactive water rapidly eliminated from CAB lenses.

A similar study was carried out with radioactive water. More than 1% was eliminated in the first 10 min followed by a slower elimination of 0.1% min. Then an increased elimination was seen for a few min. This rapid elimination was in repeated studies constantly found after 10 min. It was found in the studies with technetium and labelled leucine.

Keywords: technetium - radioactive water - leucine - elimination - contact lenses - human - in vitro

ous study on normal human persons demonstrated that the radioisotope was eliminated from soft contact lenses with 2%/min (Sørensen & Jørgensen 1980). To the best of our knowledge this was the first report on elimination of solutes from soft contact lenses placed in situ on a human eye. In the present paper these studies are extended to other types of contact lenses and the exchange of radioactive water and leucine in the lenses.

Material and Methods

Persons were selected from normal myopic persons wearing contact lenses. All were new to the lenses at the time of the determinations.

The equipment consisted of a computer assisted gamma camera. The radioisotope in the *in vivo* studies were technetium as pertechnetate in normal saline solution. A volume of this solution was instilled on the center of the contact lens while the eyelids were held apart a few seconds after the instillation. The persons blinked normally in the position under the gamma camera with the head slightly turned to the side opposite to being investigated to avoid accumulation of the radioisotope at the outer canthal area. Region of interest was the conjunctival sac area. Radioactivity was recorded at 10 second intervals and activity time curves were generated by the computer and plotted in a logarithmic system. Scintigrams were taken at the beginning and the end of the instillation. The results were presented as fractional turnover rates, i.e. slopes of the time curves. For detailed descriptions see Sørensen & Taagehøj Jensen 1977, 1979. In *in vitro* studies with contact lenses corrections for background components were not necessary.

In *in vitro* studies the lenses were pre-soaked in saline with technetium at room temperature. The lenses were then blotted and placed in 0.5 ml saline. This procedure was repeated at 2 min intervals for 98 min. The radioactivity was recorded in the washing solutions and the activity plotted in a semilogarithmic system. Straight lines were approximated by the method of least squares. In the studies with radioactive water a similar procedure was used but the study was extended using various washing volumes. The study with leucine was carried out at pH of about the isoelectric point and the other studies at near neutral pH. *In vitro* studies were made at room temperature.

Concentration of TcO_4^- in the washing solutions was counted in automatic sample counter (NaI(Tl)).

3H_2O and ^{14}C leucine was determined by liquid scintillation using Insta Gel and model 3003 Liquid Scintillation Spectrometer.

Lenses with different water content were investigated. A highly hydrophilic lens (Soflens = Duragel) and a daily wear lens (Soflens = HEMA). Thin lenses (1.3 and plano-TMA lenses with center thicknesses of 0.07 mm and 0.18 mm) were also studied. The lens (cellulose acetate butyrate) which has a very low water content was included in the study.



Fig. 1A

Distribution of technetium in a normal human eye fitted with a hard contact lens about 10-30 seconds after instillation. Radioactivity is seen in lacrimal drainage system but not in the lens.

Fig. 1B

Scintigram from the same hard lens fitted person as in Fig. 1A but 15 minutes after instillation. No radioactivity is accumulated in the hard lens. The lacrimal sac and nasal lacrimal duct are filled by tracer which has been

Results

In Figs. 1A, 1B, 2A and 2B are demonstrated the distribution of the about 0-0.5 min and 14.5-15 min after instillation. No radioactivity is accumulated in the conjunctival sac area in Fig. 1B which is from a hard lens. At the end of the determination. A similar distribution is found in a normal hard contact lens. In a soft lens fitted eye on the other hand, radioactivity is accumulated corresponding to the position of the lens (Fig. 1C). In a previous report (Sørensen & Taagehoj Jensen 1989) it was demonstrated that radioactivity was accumulated in the lens when the lens was pre-soaked with technetium before insertion as well as when the radioactive solution was instilled in the eye fitted with a non pre-soaked lens.

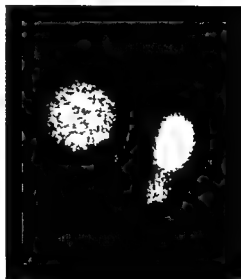


Fig 2A

Exposure of technetium in a normal human eye fitted with a *soft* contact lens. Exposure about 10-30 seconds after instillation. The tracer has not entered the lacrimal duct. Radioactivity is accumulated corresponding to the soft contact lens (in contrast to the hard lens in Fig 1A)

Fig 2B

Exposure from the *soft* lens fitted person from Fig 2A. Exposure time about 14 s. 10 s after instillation. Radioactivity is seen in the soft lens, the lacrimal sac and in the nasolacrimal duct.

The elimination curves from persons wearing hard contact lenses had a diphasic elimination as in normal eyes without contact lenses. The elimination in the first 1-2 min was very fast owing to rapid drainage of the surplus volume after instillation. A

Table 1

Elimination of technetium (TcO_4) from contact lenses. Fractional turnover rate min^{-1}

		Initial phase	Basal phase
Hard lens	(n = 5)	0.272 (s = 0.009)	0.083 (s = 0.002)
Soft lens	(n = 6)		0.020 (s = 0.004)
LS	(n = 4)	0.047 (s = 0.019)	0.004 (s = 0.003)
Scan lens	(n = 7)	0.010 (s = 0.002)	0.002 (s = 0.007)

From Sørensen & Taagehøj Jensen (1980)

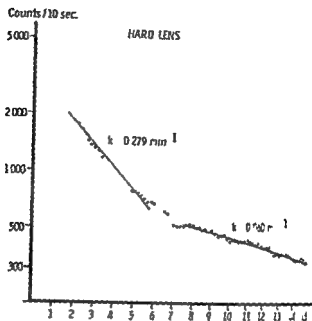
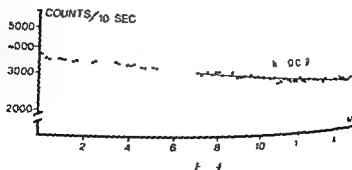


Fig 3

Activity time function curve from a hard lens fitted person. The curve has a
without contact lenses a very rapid elimination of technetium ^{99m}Tc in
in initial phase with a higher fractional turnover rate about 0.28 and
from 7.15 min

higher initial phase was found about 2.5 min followed by a slower turn
7.15 min. The results in Table I clearly demonstrate the difference
and soft lenses. In hard lens cases the elimination was in fact a two
because no radioactivity entered the lens.



Elimination of technetium (TcO_4^-) from soft lenses placed on the eye

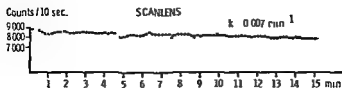


Fig 5

elimination of technetium (TcO_4) from a Scanlens[®] placed on a normal human eye

curves from persons wearing soft contact lenses (Fig. 4) were quite different from persons not wearing contact lenses. The fractional turnover rates were much lower and diphasic elimination was hardly visible for Soflens (Fig. 4) and Scanlens (Fig. 5) whereas a difference between initial and basal phase could be seen on the curves from U₃ lenses (Fig. 6). The difference was at the 6% level (t test for paired

curves). In a previous study on ordinary Soflens lenses (Sørensen et al. 1980) the fractional turnover rate for the initial phase was not calculated because the curve was almost like a straight line over the entire 15 min. The value for Soflens in Table I is the fractional turnover rate calculated in the time interval 7–15 min. The fractional turnover rate calculated in the time interval 1–7 min for a normal human eye with its microdimensions is impossible to copy in a laboratory. A very simple model might as well be as good as a refined one approximating the physiology of the human eye. We used a very simple model which demonstrated the differences between the lenses (Fig. 7). The elimination of technetium from the lenses approximated fairly well to an exponential elimination. From Scanlens lenses eliminated about 3%/min and from Soflens 1–3%/min. Plano-T had the fastest elimination (about 28%/min) (Fig. 7). The concentration of the radioisotope in the pre-soaking saline solution was extremely low and estimated to about 3

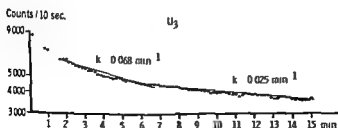


Fig 6

elimination of technetium (TcO_4) from a U₃ lens placed on a normal human eye

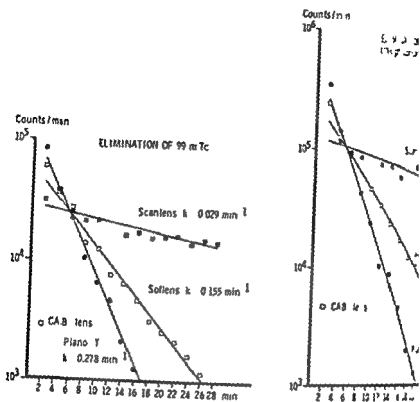


Fig 8 Elimination in vitro of technetium (TcO_4^-) from various lenses

Fig 9

Elimination in vitro of technetium (TcO_4^- high concentration) from CAB lens

pmol/l. With high concentration of technetium with most of the Tc^{99} related to Tc^{99} and a 1000 times higher concentration fractional rate the same magnitude was found (Fig. 8). The ratio Softens/Scanlens studies 19 and in the human in vivo studies 10 (Table I).

Only a small amount of technetium was absorbed by the CAB lens. radioactivity was eliminated by the first washing procedure. For the there seems to be no radioisotope other than technetium in the studies. Technetium is easily available and can be safely used for investigations.

The elimination of solutes other than technetium have to be studied in the laboratory. In this way the exchange of radioactive water was studied. The elimination of the radioactive water was found - a fraction of 11% at

/min

ELIMINATION OF $^3\text{H}_2\text{O}$
(incubated in 30 min) $T_{1/2}$ 36 sec
 k 1.12 min⁻¹Scanlens
□ Soflens
△ Plano T
○ CAB lens

Fig 9
Elimination of radioactive water. Incubation time of the lenses was 30 min

Counts/min

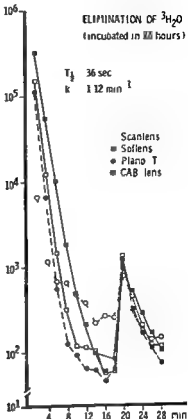
ELIMINATION OF $^3\text{H}_2\text{O}$
(incubated in 24 hours) $T_{1/2}$ 36 sec
 k 1.12 min⁻¹Scanlens
■ Soflens
● Plano T
■ CAB lens

Fig 10
Elimination of radioactive water after an incubation time of 24 h

in in the time interval 0-10 min (Fig 9). From 10-20 min the elimination was constant. After 20 min the elimination suddenly increased to a level comparable to the elimination in the 0-10 min interval. This study was carried out after incubation of the lenses in the radioactive water for 30 min. An incubation time of 24 h (Fig 10) resulted, however, in the same pattern of elimination with a slight increase after 20 min. The congruity of the curves is noteworthy, even the CAB lens, which is hydrophilic. A change of the washing volume from 0.5 ml to 5.0 ml did not influence the rate of exchange of water after 20 min in the lenses.

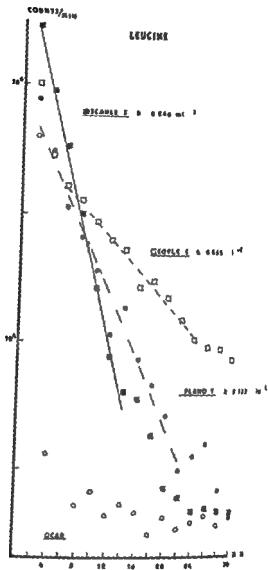


Fig 11
Elimination of leucine C₁₄

In the study with leucine Sørensen turned out to have the slowest elimination (Fig 11) with a fractional turnover rate of 0.065 min^{-1} compared to Scanlen (min^{-1}) and Plano T (0.132 min^{-1}). In the studies with pertechnetate and rat water Scanlen had the lowest fractional turnover rate. No peak with a elimination was found in the leucine study.

Discussion

It is a common assumption that soft contact lenses may act as a sustained system for drugs and solutes introduced into the conjunctival sac, only a few have been concerned with this topic. There have been several studies on it of oxygen in soft contact lens materials (Fatt 1978). The elimination of drug from soft contact lenses has been studied *in vitro* and *in vivo* in animals and patients with glaucoma (Hillman et al. 1973; van Hoose & Leaders 1974; Hillman 1974; Asseff et al. 1973; Krohn & Breidfeller 1975). These showed that the biological response to pilocarpine was enhanced by the soft lens. The *in vitro* studies revealed an initially rapid release of the drug followed by a slower release. This is fully in agreement with the theoretical prediction of a first order pattern of elimination (Richardson 1975). Using technetium as tracer, a similar initial rapid release from the lenses was found (Hansen & Kaufman 1970).

In the present study the negatively charged pertechnetate was used in the experiments on human subjects. It is noteworthy that the slowest elimination was from the most hydrophilic lens, namely Scanlens. The concentration was extremely low in the soaking solutions and in the lenses. A higher concentration of pertechnetate (proportionally more disintegrated molecules) in the soaking solution in the *in vitro* study resulted in the same fractional turnover rate (Fig. 7 and Fig. 8). In a previous paper (Sorensen & Taagehøj Jensen 1980) the same fractional turnover rate was found when lenses were pre-soaked in saline with the radioisotope and the isotope was insulated on a non-labelled lens on the eye. The radioisotope not only rapidly penetrated the lens and was very slowly released from the lens. The difference between Soflens and Scanlens is probably dependant on the difference in hydrogel material. On the other hand, the difference between Soflens and Scanlens in the human study must depend on the difference in the amount of lens material. The U.S. lens takes up relatively less radioactivity thus making the tear flow curve from the conjunctival sac with its characteristic diphasic shape visible. In the present persons this tear flow curve was seen because the hard lens did not take up the radioisotope and the curve represented the elimination of technetium from the conjunctival sac. The fractional turnover rate in persons with hard lenses cannot be converted to tear flow as in persons without contact lenses because the volume in the conjunctival sac is not the same in the two situations. The same fractional turnover rate will in a hard lens case correspond to a higher tear flow owing to the relatively higher tear volume in the conjunctival sac.

The difference between the elimination of technetium from Soflens and Plano-T lenses in the *in vitro* study can be explained by the difference in lens thickness of these lenses made of the same material. The radioisotope was eliminated more rapidly

from the thinner lens. The CAB lens probably did not take up pertechnetate. A small amount of radioactivity in the first washing solution could be the result of surface adhesion.

The simple laboratory model turned out to be in fair accordance with the *in vivo* study, since the ratio Softlens/Scanlens was 4.9 and 4.0 respectively in the studies.

The exchange of water in the lenses was found to be more than 100% *in vitro* study. In the *in vitro* study pertechnetate was eliminated 6-8 times faster than in the human study. Corresponding to this the water should be exchanged with 14.19%/min if the lenses were placed in the human eye taking no account of the difference in temperature with the subsequent decrease in pore diameter of lens material and decrease in viscosity of saline at higher temperature.

There is no obvious explanation for the elimination peak in the radioactivity study after 20 min. Though the liquid structure of water is still debated one can speculate that the dipole nature of water or hydrogen bonds could be responsible for the phenomenon. It seems, however, more reasonable that a chemical bond with water would manifest itself on the entire elimination curve and not as a single peak. If a mechanical factor was responsible one would expect the peak to be placed at a different time interval using another molecule. The study with leucine did not show a peak like water in the 30 min determination time. A somewhat similar peak was found by Hillman et al. (1975) in an elimination study of pilocarpine. After interruption of flow of eluting saline for a 15 hour overnight period, the elimination of pilocarpine from the soft contact lens had a second peak.

In contrast to the high water flow found in this study, Fatt (1973) has found that the amount of water driven through the lens was entirely negligible. The reason for these diverging results is not obvious. A relatively high diffusion coefficient for water was found by Gumpelmayer & Schwach (1972, 1973). The diffusion coefficient for water in the hydrophilic materials were found to be smaller than the diffusion coefficient for water molecules in water. They also found a great variation of the diffusion properties in the various polymers - a phenomenon which was confirmed by the present study. We found the slowest elimination of pertechnetate from the most hydrophilic material. This pattern was changed in the leucine elimination study. A similar change was found by Gumpelmayer & Schwach (1972, 1973) between glucose and amino acids.

The diffusion of a solute through a soft contact lens is not solely dependent on the molecular size. One has to consider the amount of hydration shell around the molecule, the chemical binding to the lens material and the composition of the polymer. At the present time it seems almost impossible to predict the diffusion of a solute without testing.

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CLINICAL TEAR ANALYSIS USING LASER DIFFRACTION

BY

DAVID MILLER WILLIAM J PLAUS and ROGER P ZELT

A diagnostic method has been developed to analyze tear specimens from patients with red eyes using the technique of laser diffraction. After proper tear sample preparation presence of a diffraction halo produced by the tear specimen indicates presence of an external ocular inflammation and helps rule out such conditions as glaucoma iritis and episcleritis. Measurement of the size of the halo pattern may also be used to give information as to the concentration of cells in the tear sample.

Keywords: laser diffraction - tears - tear cells - external eye disease - halo

Patients with white blood cells in their tears will report seeing a slowly developing halo if asked to look at a light source in a darkened room. The halo is first seen immediately after a blink and slowly fades until the next blink. Such halos are most vivid in acute purulent conjunctivitis and obstruction of the nasal lacrimal duct.

This phenomenon of a fading halo seen by patients with diseased eyes was first noted by Descartes in 1637 (Simpson 1953). Later Simpson, assuming it represented a diffraction phenomenon, measured the radius of the fading halo seen by his own eyes and calculated the size of cell in the tear film which produced it to be about 8μ . Simpson further suggested that since the phenomenon was described by Descartes that it should be known as Descartes corona.

Although a predicted value of 8μ is a bit small for most inflammatory reactions, the phenomenon might have clinical usefulness in screening for external eye disease in a busy clinical setting.

However, the determination of the very presence of a halo is well within the

is a demanding task for both patient and doctor in such a setting. Thus a more objective modification of the phenomenon is needed. The author suggests (Koch 1968, Wyatt 1968, Cram et al 1973, Jamieson et al 1975) that a number of cell containing biological fluids will produce diffraction patterns in the visible spectrum. Therefore we have undertaken to study the tear samples from patients with various eye diseases using a laser diffraction technique. The study further examined the relationship of these diffraction patterns to those produced by pure suspensions.

Method

Sample collection: Specimens from the lower tear meniscus were collected with fine 10 μ l capillary tubes, polypropylene disposable tips attached to a 10 μ l automatic microdispenser or directly onto the edge of glass slides. Diffraction patterns of tear samples collected by each method were evaluated for pattern sharpness.

Sample preparation: Once the tear sample was on the slide, it was noted that mucous material, cellular debris, protein aggregates or lipid globules could all obscure the cell diffraction pattern. Thus similar amounts of isotonic solutions of the proteolytic agent papain (contact lens enzyme cleaner), the mucolytic agent N-acetyl cysteine and the cytolytic agent salicylic acid, as well as a combination of the three were added to separate samples from the same patient. Diffraction patterns were then evaluated and compared with Gram or Wright stained specimens under the light microscope. In each case, an attempt

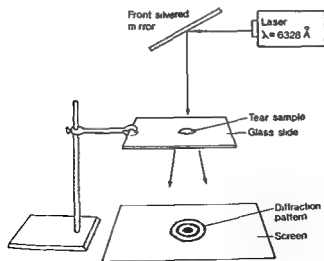


Fig 1
Apparatus used for laser diffraction of tear sample

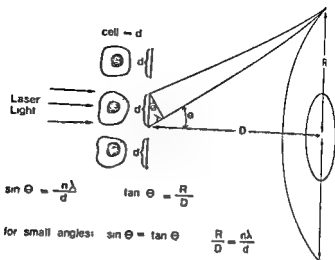


Fig. 4

Diagram illustrating the path of light waves responsible for the diffraction pattern by the cells in the tear sample

were done for each sample. Cell diameter (d) in the sample was calculated using equation $\sin \theta = \frac{n\lambda}{d}$ where n = order of the maximum measured (1 in the example above), λ = wavelength of light, d = cell diameter and $\sin \theta = R/D$ (Fig. 4).

F. Clinical trials. Once the system was made reproducible in the laboratory, tear samples were analyzed from 19 different patients with the following spectrum of external eye disease: purulent conjunctivitis, allergic conjunctivitis, vernal conjunctivitis, giant papillary conjunctivitis, contact lens induced conjunctival irritation, keratitis, ectropion, postoperative conjunctival irritation and pterygium.

Results

A. Tear Collection. Tear samples collected with the 10 μ l plastic disposable attached to the 10 μ l micropipette consistently produced the sharpest and brightest diffraction patterns.

B. Tear Preparation. Papaine was the most effective of all the additives in reducing the amount of background amorphous material seen on stain and in sharpening the diffraction patterns.

The diffraction patterns produced by the wet samples of the standard erythrocytes, neutrophils and lymphocytes more closely agreed with the accepted values.

■ than the dry samples (Bloom & Fawcett 1975). The dry samples yielded a 11% decrease in calculated cell size.

For example, the mean of 10 samples of dry preparations of the same suspension of blood cells produced a calculated cell size that was 11% smaller than from the samples when wet.

Isotonic preparations of the additives to the tear samples yielded equivalent determined cell diameters which correlated better with known cell size than did conventional pharmacologic concentrations of the additives.

Influence of Cell Concentration Table 1 shows the relationship between cell concentration (reflected in cells/mm²) and diameter of the first maxima. Tracings of the laser diffraction pattern. The samples with an asterisk are from tears; those without are from neutrophil suspensions isolated from blood. Fig. 3 is a graphic representation of the laser diffraction pattern (Fig. 2).

Influence of Cell Type Using the laser diffraction technique, 9 samplings of a blood cell specimen yielded a mean first maxima diameter (2R) for a red cell suspension of $3.3 \text{ mm} \pm 0.15 \text{ mm}$ for a lymphocyte suspension of $4.95 \text{ mm} \pm 0.10 \text{ mm}$ and for a neutrophil suspension of $3.49 \text{ mm} \pm 0.10 \text{ mm}$.

The laser diffraction pattern (Fig. 2) is composed of a central bright red light (central maximum) surrounded by ever widening red circles (different orders of maxima) separated by dark circles (different orders of minimum).

Clinical Findings The dilution studies in combination with the haematocytometer showed that a tear sample must have at least 12 cells/mm² or about 1 or 2 cells per high power field in order to produce an easily measurable laser diffraction pattern. Clinically speaking, conjunctival inflammations of trace to 1+ reaction will

Table 1
Cell concentration vs Halo size

Neutrophils/mm ²	(2R) Diameter of First Maxima (mm)
400	8.2
86	3.8
54	3.5
44	3.3
18	3.3
12	3.1

* Neutrophils in clinical tear sample

Table II
Clinical Diagnosis vs. Average Size Cell in Tears

Clinical Diagnosis	Calculate Average Tear Cell Size \pm SD	Microscopic Appearance of Tear
1 Allergic Conjunctivitis	$7.3 \mu \pm 0.3$	0+ Lymphocytes occasional squamous cells
2 Vernal Conjunctivitis	$8.1 \mu \pm 0.3$	4+ Lymphocytes rare squamous cells
3 Chronic Purulent Conjunctivitis	$9.2 \mu \pm 0.3$	3+ neutrophils 1+ lymphocytes 1+ squamous cells
4 Acute non-traumatic purulent conjunctivitis	$11.0 \mu \pm 0.3$	3+ neutrophils 1+ lymphocytes
5 Acute purulent Conjunctivitis (1 day after ectropion repair)	$11.6 \mu \pm 0.3$	2+ neutrophils 1+ lymphocytes
6 Contact Lens Irritation	$10.2 \mu \pm 0.5$	3+ squamous cells

usually not produce enough inflammatory cells in the tears to give a mean pattern. Thus conjunctival reactions of 2+ to 4+ would yield the best patterns.

Is there any way of determining the predominant cell type in a tear specimen using this technique? Arakau (1966) is working with red blood cell samples and that if cell concentration is known cell size can be approximated by the proper conversion factor. Thus if we make the assumptions a) of a very high concentration (200-400 cells/mm²) and b) that the cells may arrange themselves in a uniform mesh we may take the calculated cell size (d) of equation (1) and multiply (d) by 2. The resulting value suggests the diameter of the major cell present in the sample.

Table II relates the calculated cell size using laser diffraction with the stained appearance of the tear specimen of high concentration as seen under a microscope for some representative cases.

DISCUSSION

The results of this study support the basic premise of Simpson that cells in the tear film caused by external ocular inflammation are responsible for the presence of

ites halos. The study further demonstrated that these halos could be produced in vitro in a rapid (1 min) procedure. Thus one might foresee the when a patient with a red eye appears in a busy hospital emergency room, a history is taken, a nurse or trained technician takes a tear sample and performs a laser diffraction test. The presence of a halo from the specimen may rule out a diagnosis of iritis, glaucoma or episcleritis for emergency room technician on duty.

The question of the mechanism of the halos requires further discussion. In a very real way, Fig. 4 illustrates the events which produce the diffraction pattern. The question (Miller & Benedek 1973)

$$\sin \phi = \frac{n\lambda}{d} = \frac{R}{D} \quad (1)$$

where n = order of maxima

λ = 6328 mμ (wavelength of He-Ne laser light)

R = radius of first maxima

D = distance of tear sample to screen

d = cell size

describes the angular radius of a halo produced by a grating or mesh. Thus one may say that a meshwork of biological cells are related to a diffraction pattern in a similar way.

However, the equation assumes a) theoretically optimal spacing between cells of all sizes of about twenty times the wavelength of light, b) a significant difference in index of refraction between cell and surrounding media (i.e. tears and air), c) uniform cell size, d) uniform cell shape, e) uniform orientation of cells, f) even cell distribution. Obviously, our tear samples do not meet these criteria. Be that as it may, can any further information be gained from the diffraction pattern?

In 1966 Krakau, working with blood samples of different concentration, faced the same dilemma. By assuming that the red cells behave like opaque discs, he was able to relate red cell size to the first maxima in very sparse cell preparations and cell concentration to the first maxima in dense preparations. Our results with different concentrations of neutrophils (Table I) also supports the concept that a relation between cells exists which changes with the spacing between the cells. With a high cell concentration, it is the size of the cell clumps themselves which determine the diffraction pattern. However, with low cell concentrations, the spacing between the cells becomes comparable to the size of the cell, and the radius of the first maxima is related to the cell size itself, as in equation (1).

Therefore, a few generalizations concerning cell concentration can be made from

the measurement of the diffraction pattern of a clinical tear sample and equation (1)

Concentrations of cells below 10 cells/mm² will probably be too sparse to produce a pattern

Concentrations of between 10 to 100 cells/mm² allow enough spacing between cells so that inserting the radius of the first maxima of the halo into equation (1) and calculating d results in about 10 μ

Concentrations of cells above 100 cells/mm² produce a tighter network and insertion of the radius of the first maxima of the halo into equation (1) and calculating d results in a calculated cell size of about 5 μ

In sum, a tear sample yielding a laser diffraction pattern suggests an ocular inflammation with an inflammatory cell concentration of at least 10 cells/mm². If the radius of the halo is measured, inserted into equation (1) and the value for d results in about 10 μ , then the cell concentration is between 10 cells/mm². If the resultant d equals about 5 μ , the inflammatory cell concentration is over 100 cells/mm².

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LACRIMAL PATHOLOGY EVALUATED BY DYNAMIC LACRIMAL SCINTIGRAPHY

BY

T. SØRENSEN and F. TAAGEHØJ JENSEN

This study of pathological lacrimal systems demonstrated the usefulness and sensitivity of dynamic lacrimal scintigraphy (dynamic use of computer assisted gamma camera). The method was very sensitive: even small lacrimal obstructions caused a distinct change of the outflow curves. This technique complements other tests in lacrimal assessment especially in patients with epiphora and normal conventional tests.

Key words: lacrimal pathology - dynamic lacrimal scintigraphy - human - technetium - gamma camera

urate pre-operative assessment of the lacrimal drainage system is necessary and includes the demonstration of anatomical defects as well as the functional capacity of the lacrimal drainage system. This latter part of the assessment has in recent years been possible by lacrimal scintigraphy. In these studies on lacrimal scintigraphy the evaluation has been carried out by comparing scintigrams at various intervals after instillation of the radioisotope or by measuring transit times to various parts of the lacrimal drainage system.

In previous papers (Sørensen & Taagehøj Jensen 1977, 1979) we have described the technique of "dynamic lacrimal scintigraphy" (i.e. outflow studies by dynamic use of computer assisted gamma camera) and presented the results of these studies in normal human subjects. The amount of conjunctival transport in patients with defects in drainage systems and in normal persons was also reported.

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In the present study the dynamic lacrimal scintigraphy has been performed in patients with pathological changes of the lacrimal drainage system

Material and Methods

Patients attended to the Eye Department of Århus Kommune hospital and during a two years compliance of epiphora were included in the study. Those who underwent a complete lacrimal obstruction were excluded since these results have been presented in a previous paper (Sørensen & Taagehøj Jensen 1979).

The method was described in detail in the papers mentioned above. It is noteworthy to emphasize the importance of the head tilt opposite to the eye being investigated as a correct method of instillation with a manual fixation of the eyelids a few seconds after instillation.

All investigations were carried out with the patient in the supine position. It is preferred owing to the steady fixation obtained in a plaster bandage during the 15 min recording time. Without the head tilt in the supine position tears accumulate in the canthal area (Sørensen & Taagehøj Jensen 1979) but with the head tilt no accumulation is seen and no difference in tear flow between supine and upright position was demonstrated.

A volume of 10 µl of a normal saline solution containing technetium as pertechnetate (^{99m}Tc) as instilled on the center of the cornea. Scintigrams were taken at 0, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, 1490, 1500, 1510, 1520, 1530, 1540, 1550, 1560, 1570, 1580, 1590, 1600, 1610, 1620, 1630, 1640, 1650, 1660, 1670, 1680, 1690, 1700, 1710, 1720, 1730, 1740, 1750, 1760, 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Counts/10 sec (log scale)

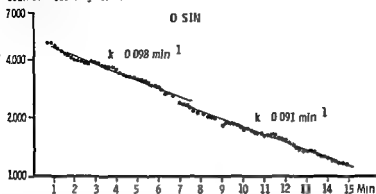


Fig 1A

Curve from patient 1 left eye. Schirmer tear test was 2 mm/5 min. The surplus is rapidly eliminated from 0–1 min. A biphasic elimination is not present in this eye with reduced tear secretion. The slope of the curves k is the fractional turnover rate.

Counts/10 sec (log scale)

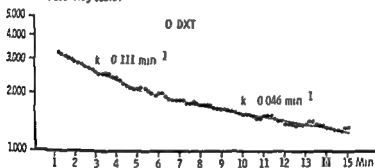


Fig 1B

Curve from patient 1 right eye. A biphasic elimination is seen with a shift after 5–7 min in this eye with the higher Schirmer tear test (4 mm/5 min).

lution by the instillation procedure. The patient's left eye (Fig 1A) was thus unable to mount a stimulated tear secretion whereas a slightly higher initial phase was found in the right eye. The patient had not experienced dry eye symptoms. The very rapid elimination in the first min caused by the rapid drainage of the surplus volume of tears after instillation of 10 μ l technetium solution.

Counts/10 sec. (log scale)

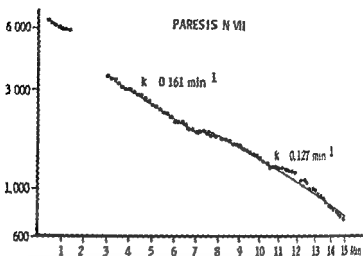


Fig 2A

Outflow curve from right eye patient 2 with epiphora owing to incomplete remission facial palsy. The rapid elimination of the surplus volume after installation of instillation is a biphasic elimination with a shift after 6-7 min is present but not distinct.

Counts/10 sec. (log scale)

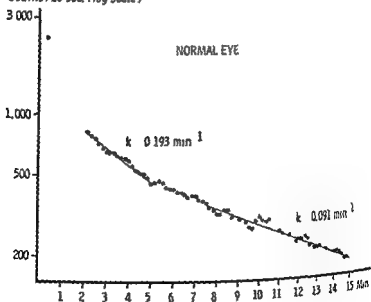


Fig 2B

Tear flow curve from patient 2's normal eye

1 pump insufficiency

a 63 year-old man had a peripheral left facial nerve paresis of unknown origin with a gap of the left palpebral aperture but no lagophthalmus. The Schirmer tear test was 20 mm in 5 min. The dynamic lacrimal scintigraphy was normal in the unaffected eye (Fig. 2B). On the paretic side showed a reduced ability to pass the purpuric volume after 2 min down the drainage system in the first 2 min (Fig. 2A).

Spasmodic blinking

A 72 year-old man referred to the hospital for dry eye syndrome. The Schirmer tear test was 0 mm in 5 min in both eyes. Slit lamp examination was normal with a normal lacrimal sac and no vital staining with Rose Bengal. The patient suffered from spasmodic blinking which was completely normal (Fig. 3) with a biphasic shape and a rapid elimination 0-2 min after instillation. This patient was apparently blinking the tears down the drainage system thus avoiding absorption of the tears into the filter paper. After 15 min tracer was found in the tear sac and nasolacrimal sac but no tracer entered the nose.

Counts/10 sec. (log scale)

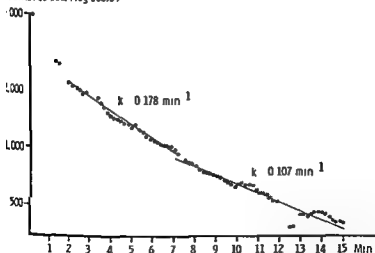


Fig. 3

Flow curve from patient 3 who suffered spasmodic blinking. The curve is normal though the Schirmer tear test was 0 mm in 5 min.

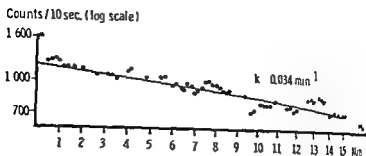


Fig. 4

Tear outflow curve from patient 4. Pre sac stenosis owing to radiation for rodent ulcer in the inner canthal area

Canalicular disease

Patient 4 a 50 year old man with atrophic skin and indurated subcutis in the medial canthal area owing to radiation for rodent ulcer. This patient had epiphora and dacryoscintiscinography showed a reduced tear outflow with no initial phase (Fig. 4). Small amounts of radioactivity were seen in the nasolacrimal duct after 15 min. The lower canaliculus was completely occluded and the upper had a stenosis which was dilated with some success.

Calculus in the upper canaliculus

Patient 5 a 60 year old woman with epiphora. Tear flow was reduced and the tear flow curve was practically monophasic (Fig. 5). Small amounts of radioactivity was seen in the lacrimal sac. The drainage system was filled with mucus and a calculus was found in the upper canaliculus.

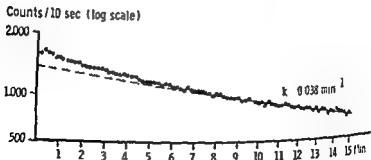


Fig. 5

Tear outflow curve from patient 5. Calculus in the upper canaliculus and small amounts of mucus in the drainage system

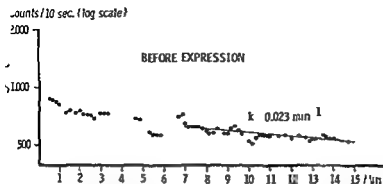


Fig 6A

outflow curve from patient 6 who had a complete obstruction of the nasolacrimal duct. The curve was found when the drainage system was filled by mucus before expression of the lacrimal sac

6 a 71 year-old man who had a fracture of the maxillary bone with subsequent occlusion of the nasolacrimal duct and slight distension of the lacrimal sac. Dynamic lacrimal scintigraphy was carried out before (Fig 6A) and after (Fig 6B) expression of mucus from the lacrimal sac. The scintigram in Fig 6C was exposed 14–15 min after instillation of the isotope. The lacrimal sac was incompletely filled by the tracer. After expression of the sac a new instillation of technetium resulted in a good filling of the lacrimal sac (Fig 6D).

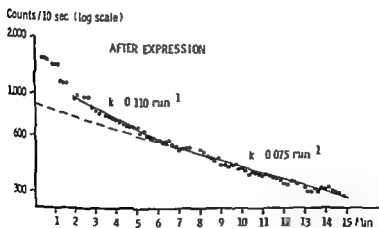


Fig 6B

curve from patient 6 after expression on the lacrimal sac. A more rapid elimination is seen with a biphasic shape as in normal persons.

THE OCULAR RESPONSES OF ORAL ADMINISTRATION PENBUTOLOL IN THE GLAUCOMATOUS PATIENT

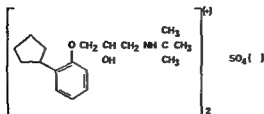
BY

G K KRIEGLSTEIN E GRAMER and W LEYDHECKER

A single oral dose of 20 mg or 40 mg Penbutolol was given to two groups of untreated glaucoma patients. The drug significantly decreased intraocular pressure and was dose related. The IOP response was paralleled with decrease in pulse rate without significant effect on blood pressure. In another group of 27 glaucoma patients which were under sufficient IOP control with topical treatment with different antiglaucomatous agents the daily peroral treatment with 40 mg Penbutolol did not result in a further decrease of IOP. However, a significant drop in pulse rate could be noted over the four week period of treatment in this series of patients. Blood pressure was similar to the single dose study, not significantly affected. Penbutolol treatment did not significantly change pupillary diameter, quantitative tear flow or corneal sensitivity. The potential usefulness of the drug in glaucomas with systemic hypertension or as additive treatment when topical treatment is insufficient is outlined.

Keywords: glaucoma, open angle - β blockers, Penbutolol - oral therapy, intraocular pressure

Penbutolol is a new promising β blocking agent which in the treatment of systemic hypertension has been found to be superior to Propranolol both in β -block potency and duration of action (Boksay 1979). Chemically this drug is a 1-isomer of a phenoxyalkylamine derivative (() 1 tert butylamino 3 (9-cyclopentylphenyl) 2 Propanol sulfate, Fig. 1). Pharmacologically Penbutolol can be characterized as follows. The β blocking potency proved to be 4.5 times that of Propranolol experimentally and clinically. It has only little intrinsic sympathomimetic activity, about 50% that of Alprenolol. The drug produces little membrane stabilizing activity and has no cardio selectivity.



Penbutolol

Fig 1

The molecular structure of Penbutolol sulphate

ce betablockers are nowadays widely used in international medicine and ally as antiglaucomatous drugs in ophthalmology there is a frequent therapeutic link between both fields. Systemic betablocker therapy can affect the ophthalmic eye in different ways. It may unfavorably influence arterial perfusion pressure at the level of the optic nerve head. It may represent a combined therapeutic approach in ocular and systemic hypertension. It may mediate chronic ocular subsensitivity and/or produce dry eye syndromes. The elucidation of part of these questions was the aim of the present study.

Methods

The present investigation was subdivided into two series of clinical studies. The first was devoted to the acute effects of peroral administration of Penbutolol; the second one to the chronic effects of the same drug.

clinical studies Series I

This series comprised two groups of open angle glaucoma patients (ten patients in each group). The diagnosis of chronic open angle glaucoma was based on an untreated intraocular pressure level of more than 25 mmHg and/or glaucomatous disc changes and/or glaucomatous visual field loss. Two of the above parameters were defined as necessary for diagnosis. None of the patients had received betablocker therapy in the recent two months. Miotic and adrenergic antiglaucomatous treatment was continued prior to the test for at least 48 h. In all patients no other anterior segment pathology other than glaucoma was present; there was no history of glaucomatous surgery in these patients. Goldmann applanation tonometry

(same instrument, same observer throughout the study) was performed at 4 h, 6 h, 8 h and 24 h after oral administration of 20 mg (first group of ten patients) or 40 mg (second group of ten patients) Penbutolol. Blood pressure (sphygmomanometer according to Riva Rocci) and pulse rate (using a stop watch) had been measured in the same intervals. Pupillary size (at the Goldmann perimeter standard light conditions) and quantitative tear flow (using Schirmer test) were recorded prior and 2 h, as well as 4 h after drug application.

Clinical studies: Series II

Fifty-three of 27 glaucoma patients had been investigated in this series. The criteria for the diagnosis of chronic open angle glaucoma had been the same as in series I. However, the IOP of the patients of series II were controlled (≤ 19 mmHg) either by topical antiglaucomatous treatment or by a successful antiglaucomatous surgical procedure. Patients with local or systemic betablocker therapy over the past 6 months were carefully excluded. The following parameters were recorded (before Penbutolol administration) on day 14 and day 28 of Penbutolol 75 mg per day: Intraocular pressure, blood pressure, pulse rate, tear flow and corneal sensitivity (using the nylon monofilament aesthesiometer of Lunau Co., Bonnet-Frohnhauser/München). Corneal sensitivity results were expressed as pressures of g/mm² to induce blinking reflex. Duplicate readings were made in each measurement; the arithmetic means considered for statistics.

Paired *t* test was used to decide on the statistical relevance of the results.

Results

Series I

The effect of a single oral dose of Penbutolol on the intraocular pressure of groups of ten glaucoma patients is demonstrated in Fig. 1. 20 mg and 40 mg Penbutolol decreased IOP significantly. The untreated pressure levels were 28.3 mmHg on the average in the two groups. The mean IOP response after 20 mg Penbutolol p.o. was 8.05 mmHg (according to 29% relative pressure reduction). After 40 mg Penbutolol p.o. (equivalent to a relative IOP decrease of 40.7%). The maximum of the response occurred after 4 h; the effect was significant after 8 h. However, after 24 h post treatment the IOP did not differ from the pre-treatment levels. The drug response of the two dosages appeared to be significantly different, confirming a dose-response relationship. Blood pressure was not affected in a statistically significant manner with either 20 mg or 40 mg.

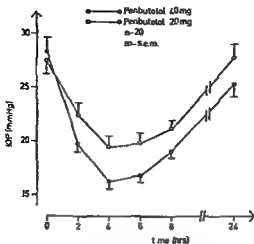


Fig 2

mean time course of the IOP response of a single oral dose of 20 mg and 40 mg Penbutolol in two groups of ten glaucoma patients. The abscissa gives the time after drug application the ordinate the IOP. Arithmetic means and standard errors of the means are presented.

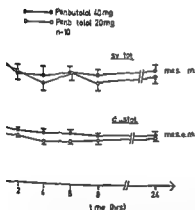


Fig 3

effect of a single oral dose of 20 mg and 40 mg Penbutolol in two groups of ten glaucoma patients on systolic and diastolic blood pressure. The abscissa gives the time course after drug application the ordinate the blood pressure. Arithmetic means and standard errors of the means are presented.

Fig 4

mean time course of a single oral dose of 20 mg and 40 mg Penbutolol on pulse rate in groups of ten glaucoma patients (abscissa time after drug application ordinate pulse rate). Arithmetic means and standard errors of the means are presented.

Penbutolol There was a tendency towards a moderate systolic pressure decrease which could not be confirmed by statistics. Blood pressure effects over the observation period are illustrated in Fig. 3.

With both Penbutolol doses a significant drop in pulse rate could be noted after drug application which remained significant for 8 h. After 24 h there was some pulse rate reduction but this was not of statistical relevance (Fig. 4).

Pupillary diameter exhibited no significant drug response with either Penbutolol (Fig. 5).

This single dose study of series I did not verify an effect of Penbutolol on tear flow as measured by the Schirmer test.

Using 40 mg Penbutolol orally a slight decrease in tear flow was noted on which could not be separated from reading error (Fig. 6).

Series II

The mean IOP in the 53 treated glaucomatous eyes of this series before Penbutolol administration was 19.3 mmHg. Additive treatment with 40 mg Penbutolol over 4 weeks resulted in a further decrease of IOP of 1.3 mmHg which was statistically significant (Fig. 7).

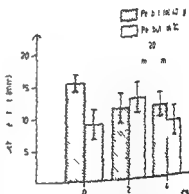
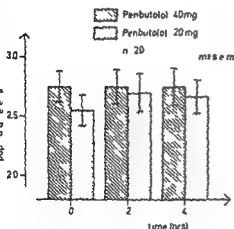


Fig. 5

The effect of 20 mg and 40 mg Penbutolol orally on pupillary diameter. The abscissa: the time after drug application; the ordinate: the pupillary diameter (arithmetic means and standard errors of the means).

Fig. 6

The effect of 20 mg and 40 mg Penbutolol on quantitative tear flow. The abscissa: the time after drug application; the ordinate: the wetting distance as indicated by Schirmer. The columns indicate arithmetic means; the vertical bars: the standard errors of the means.

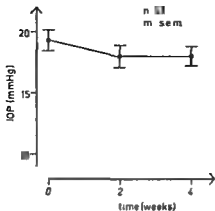


Fig 7

In IOP response of a 4 weeks treatment of 27 glaucoma patients (53 eyes) with 40 mg Penbutolol daily (arithmetic means and standard errors of the means)

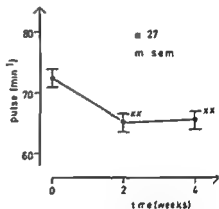
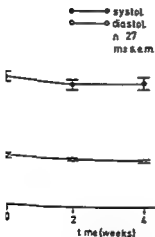


Fig 8

Effect of add-on Penbutolol treatment (40 mg per day) in 27 glaucoma patients on systolic and diastolic blood pressure. The abscissa gives the time of treatment, the ordinate the blood pressure (arithmetic means \pm standard errors)

Fig 9

Effect of Penbutolol treatment over 4 weeks on pulse rate in 27 glaucoma patients. The abscissa gives the time of treatment, the ordinate pulse (arithmetic means \pm standard errors). XX means statistically significant at the 0.01 level

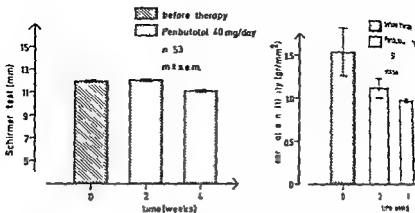


Fig 10

The effect of Penbutolol treatment on quantitative tearflow as measured by Schirmer (column with oblique lines before treatment, columns with dotted pattern after treatment). The abscissa gives the duration of treatment, the ordinate the wetting distance in 5 min test.

Fig 11

The effect of Penbutolol peroral treatment on corneal sensitivity in 21 eyes of 6 patients. Corneal sensitivity is expressed in pressure per square millimeter to trigger reflex. The column with oblique lines gives the parameter before treatment, the columns after treatment. The vertical bars indicate the standard errors of the test.

Systolic and diastolic blood pressure (mean baseline level 134 mmHg systolic, 84 mmHg diastolic) were not essentially changed over the 4 weeks period of Penbutolol treatment in these patients. However, a considerable effect, highly significant, could be identified in pulse rate. The pre-treatment pulse rate was 79 min^{-1} and 4 weeks after treatment 65 min^{-1} (Fig 9). Schirmer test before, 2 and 4 weeks after Penbutolol treatment revealed no effect of the drug on tear flow. The wetting distance on the filter paper was essentially unchanged and about 11 mm. The evaluation of corneal sensitivity showed a tendency towards increase of sensitivity during the treatment phase. However, because of the fairly high readings and this semi-quantitative technique the differences were not significant (Fig 11).

Discussion

The results of the present study indicate that a single oral dose of Penbutolol is capable of reducing intraocular pressure in untreated glaucomas in a dose-dependent, significant way. The magnitude of pressure reduction turned out to be significant.

most effective topical antiglaucomatous drugs commonly used in clinical

The time course of the IOP response was paralleled with a decrease in rate without a significant effect on blood pressure. This cardiodepressive effect of the drug can be of crucial importance for the disease process when the glaucomatous damage of the optic nerve head is mixed with anterior ischemic pathology of the papilla. In this respect Ohlstrom & Pandolfi (1978) reported an increase of glaucomatous field loss with long term treatment of glaucoma with oral Propranolol in five out of 17 patients. In another paper the same author (Ohlstrom 1973) recommends use of a dose of the systemic β blocker which is sufficient for blood pressure. Since we know that peripheral ischemic conditions constitute contraindications for β blocker therapy (Dietz et al 1978) the dissociation of IOP response and blood pressure response by dose appears advisable. In patients with unsufficient IOP control by traditional antiglaucomatous topical treatment systemic β -blocker therapy can add the necessary pressure reduction and prevent glaucomatous nerve damage without the concern of lowering arterial perfusion pressure in the tissues which are at risk. This combined effect of miotics and β -blocker was extensively established by Wettrell (1977) using Propranolol and Atenolol. If we consider that the maintained long term IOP reduction of β blocker is about half of the acute response, the aspect of combined treatment receives even more support (Drance 1970; Kriegstein 1978, 1979). The effect during the 4 weeks follow up under Penbutolol treatment was insignificant because the pressure in these patients was fully under control by other antiglaucomatous therapy. The decrease in pulse rate and the lack of significant increase on blood pressure was in agreement with the single dose study. Neither in single dose nor in multiple dose investigations could we identify a significant effect on quantitative tear flow by the Schirmer's test. There are experimental data that β_1 receptors are involved in the regulation of tear flow (Gill et al 1979) and there are clinical data confirming transitory manifestations of dry eyes in the long term treatment with β blockers (Nielsen & Eriksen 1979) that is of great parameter interest in β blocker glaucoma therapy. A more careful follow up over longer periods of time in elderly glaucoma patients who are borderline with tear flow capacity will shed further light on this problem. In all other β blocker studies in ophthalmology an influence of the drug on pupillary diameter could not be verified. There is in fact β -adrenergic innervation of the sphincter muscle of the pupil in the primate eye (Zimmermann & Frerking 1979) which in the human is obviously not relevant compared to a cholinergic and cholinergic tonus of the iris. Penbutolol has little membrane stabilizing activity. Nevertheless a decrease in corneal sensitivity due to chronic submaximal anaesthesia could not be confirmed by the semi-quantitative technique applied. In contrast a tendency towards an

increase in sensitivity was observed and it is speculative that subliminal work may result clinically in over sensitization. Using a prototype instrument for tonometry in quantitative manner Buhr & Draeger (1979) found a significant sensitivity of the cornea in topical β blockers and even in those which are supposed to have no membrane stabilizing activity such as Timolol (Zimmerman & Kaufman 1977).

Looking back to the initial questions raised in the introduction one may conclude in the light of the present results that Penbutolol may represent a useful local glaucoma therapy in patients who require systemic β blockers for medical reasons. Penbutolol could possibly also be of value in patients who need additional reduction on top of their local antiglaucomatous regimen. Further clinical evaluation is warranted to establish the maintained effect of Penbutolol versus untreated glaucomas.

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A MULTICENTRE STUDY OF THE EFFECT AND TOLERANCE OF OCUSERT P 40

BY

T ÅKERBLOM E AURELL² J CRISTIANSSON³ V KRIISA KUNNOS⁴
and O WIEBERT⁵

The suitability of treatment with Ocuser[®] P-40, a lamellar system inserted in the eye and allowing the constant release of pilocarpine, was studied in a group of patients (age range 43-83 years) with wide angle glaucoma. Those patients completing a 3-week trial were included in an 8-month follow-up study in which the long-term efficacy and tolerance of Ocuser[®] were studied. Treatment was discontinued in 14 of the 42 patients in the 3-week trial owing to ocular irritation and retention problems (n = 11) or unsatisfactory control of the intraocular pressure (IOP) (n = 2). Of the 29 patients entering the long-term study, two were withdrawn because of tolerance problems and two as a result of unsatisfactory control of IOP. The 25 patients completing the study considered the Ocuser[®] system more convenient and less liable to produce troublesome side-effects than their previous therapy. Ocuser[®] was best tolerated by the younger patients.

Key words: glaucoma, intraocular pressure, Ocuser[®], pilocarpine.

Pilocarpine has been used to reduce the intraocular pressure in glaucoma patients about 100 years. The substance is generally administered in the form of drops. Optimal treatment with eyedrops usually entails frequent administration. Patients therefore have difficulty in complying with the treatment (Speath & Vincent 1972). Pilocarpine drops also cause side effects, above all in the form of miosis and myopia. These reactions occur shortly after administration and are a temporary high concentration of the drug in the eye. Ocuser[®] P-40 has

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been developed in attempt to overcome these problems. It consists of an elliptical lamella which is placed in the upper or lower fornix in contact with the tears. pilocarpine is released at a constant rate over a 7-day period except in the first few hours when the rate of diffusion is higher.

The Ocusert® system has been shown in clinical studies to produce a considerable reduction in intraocular pressure (IOP) over 7 days (Fraunfelder et al 1974, Quigley et al 1975). The product also allows the same effect to be obtained with a smaller amount of drug compared with administration in the form of drops (Armaly & Rao 1973). Together with the continuous release this has meant a considerable reduction in the side effects produced by pilocarpine. The incidence of myopia and miosis has been consistently low (Place et al 1972).

Certain patients experience initial difficulties in inserting and wearing the lamellae (Henning 1976) although with thorough information and training these problems can be reduced. It is unlikely, however, that all these patients will tolerate the lamellae. The aim of this investigation was to determine which patients are best suited to using Ocusert®. The patients who comprised the first part of the study were subsequently kept under observation in order to assess the long term effects of Ocusert®.

Material and Methods

The Ocusert® system

The Ocusert® developed by the Alza Corporation in the USA, comprises three membrane layers (Fig. 1). The middle membrane consists of pilocarpine bound to a polymer and is placed between two layers of ethyl vinyl acetate which determine the rate of diffusion of drug from the system. In contact with the tears, pilocarpine is released at a constant rate of $3 \mu\text{g/h}$ with the Ocusert® P 20 and $40 \mu\text{g/h}$ with the Ocusert® P-40 (which was used in this study) over a 7-day period. During the first 19 h, however, the rate of diffusion is higher and this is known as the burst effect.

Patients

Only patients previously treated with miotics for open angle glaucoma were included in the study. Some patients were also receiving adjuvant treatment with adrenaline or acetazolamide. A condition of inclusion was that the patient's IOP was well controlled with conventional treatment.

Forty-two consecutive patients with a mean age of 63 years (range 43-83 years) were selected for the study. At the start of the study 34 patients had excentric papilla and/or visual field defects. Eight patients had an abnormal IOP but normal papilla and no detectable visual field defects. These patients were receiving prophylactic treatment for ocular hypertension. All patients had previously been

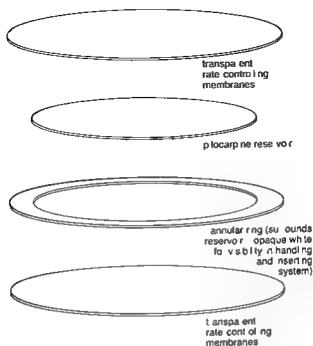


Fig 1
The Ocuseri® system

d with miotics for at least one month (mean 28 months). Thirty-eight patients using pilocarpine solutions of varying strength (1-4 %) and twelve were also using adjuvant treatment with adrenaline or acetazolamide. This treatment was used unchanged during the study except in one patient who stopped using saline owing to an allergic reaction. Two patients had previously been treated with carbacholine and the remaining two had used physostigmine and demecarium and adrenaline respectively.

Term study
Patients were given detailed information about the aims and procedures of the study before being included in the trial. After general examination of the eye, the visual field was investigated by means of a Goldmann perimeter and the intraocular pressure was determined using a Goldmann applanation tonometer. Measurement of IOP was performed on average 2 h after the last instillation of miotic drops.

Miotic treatment was then discontinued and the patient was given detailed and written instructions about the use of the lamellae. The patient was given opportunity to practice inserting and removing the lamella. The patient was for examination after 3, 9 and 21 days at which times the IOP and tolerance to Ocuser[®] was recorded.

Long term study

The patients who completed the short term study were subsequently admitted to follow up study, the IOP and tolerance being recorded after 4 and 8 months treatment. The patient's visual field was investigated before the start of treatment and after 4 and 8 months.

Results

Short term study

Substitution of Ocuser[®] for pilocarpine drops was achieved without any initial problems or changes in IOP in 29 of the 42 patients. These 29 patients were subsequently included in the long term follow up of Ocuser[®] P-40 treatment.

One patient discontinued the study as he was not capable of maintaining medication with either drops or Ocuser[®]. Two patients were withdrawn from study owing to unsatisfactory pressure control. The remaining 10 patients who dropped out had so much difficulty tolerating the treatment that they preferred to revert to their previous treatment. In most cases this happened during the first few days of treatment. The main problems were that the patients dropped the lamellae or found them irritating to their eyes.

Long term study

Of the twenty nine in the long term follow up study, one was an alcohol addict and discontinued the treatment after one month. Three patients reverted to their previous treatment (pilocarpine 2%, pilocarpine 4% and physostigmine 1% respectively) as their IOPs rose. One of these patients also exhibited progressive visual field defects after three months.

The remaining 25 patients completed 8 months treatment. Two patients were given adjuvant therapy with pilocarpine and adrenaline drops respectively. Their IOPs tended to rise. As the follow up period was largely a tolerance study, only two patients were still included in it. No significant change of the IOP was registered in the 25 patients during the 8 months of the study (Table I) nor was any change in the visual field detected in these patients.

Table I

Mean I O P in the 20 patients who completed both parts of the study

Treatment	Previous treatment	Ocuser [®] P-40 system				
		4 days	7 days	3 weeks	4 months	8 months
Initial time						
Number of eyes	40	40	43	40	40	40
mmHg	20.9	21	21.3	20.8	21.9	21.4
	0.66	0.53	0.50	0.54	0.58	0.59

most common side effect of previous treatment was altered vision after use of miotic drops. Fourteen patients considered this troublesome (Table I). These side effects declined markedly within one week of the start of Ocuser[®] treatment.

During the first few days of treatment with Ocuser[®] many patients dropped the lamella. This problem was probably partly due to the lamella being placed in the conjunctival sac. Initially the patients were recommended to insert the lamella in the lower fornix but with time most patients found it easier to use the upper fornix. The problem therefore decreased as the patients became accustomed to treatment.

Table II

Side effects reported by the 20 patients who completed both parts of the study

Treatment	Previous treatment	Ocuser [®] P-40 system				
		4 days	7 days	3 weeks	4 months	8 months
Initial time						
Conjunctival injection	2	3	2	3	4	6
Increased secretion	3	5	2	4	3	3
Altered vision	14	2	1	1	0	0
Lamella dropped						
0		12	14	14	11	16
1-2		12	7	8	7	3
3-4		1	3	1	3	3
≥ 5		0	0	0	3	3

Conjunctival injection

One patient reported discharge of pus from the eye during one period

One patient did not state the number of lamella dropped

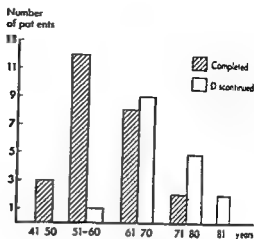


Fig 2

Age distribution of patients who completed discontinued the study

All 25 patients who completed the study wished to continue on Ocuser[®] treatment as they experienced little or no alteration in their vision, in comparison to their previous treatment with miotic eyedrops. They also found the Ocuser[®] lamella more convenient and experienced a greater sense of freedom as they no longer needed to have their bottle of pilocarpine drops constantly within reach.

As shown in Fig 2 the younger patients had no difficulty in switching to the Ocuser[®] system. The change of therapy caused increasing problems with increasing age.

Discussion

Treatment with pilocarpine eyedrops often causes side effects in the form of transient myopia and miosis. However, both patients and doctors have accepted these side effects up to now as being inevitably linked with pilocarpine treatment. The theoretical superiority of the Ocuser[®] lamella is therefore readily accepted, particularly by younger patients. Compared to pilocarpine eyedrops, the Ocuser[®] lamella requires somewhat greater manual dexterity. Initially, the patient must accept and endeavour to tolerate the feeling of having a foreign body in the eye. The above factors may limit the number of glaucoma patients who are suited to this form of treatment.

The results of the short term study also show that the patient should be given advice and encouragement during the first few weeks of treatment in order to

any initial problems. This requires a positive attitude on the part of the patient. Many patients will otherwise be deprived of the benefits of the treatment. Ocusert® lamella may with advantage be combined with other glaucoma therapy, both as drops and as tablets. In this situation too the patient will tolerate minor improvements in the treatment schedule. The change-over from previous treatment (most patients were previously treated with pilocarpine solutions 2-4%) to Ocusert® generally caused little or no change in IOP in those patients who could tolerate the Ocusert® system. The pilocarpine concentration in the lamella may therefore be considered sufficient for many patients. Although 13 patients dropped out of the initial 3 week study, in only two cases was this due to inadequate control of IOP. During the long term study a further three patients reverted to treatment with drops for this reason, and in two cases supplementary treatment with drops was necessary. The long term study has shown the Ocusert® lamella to be a valuable therapeutic alternative for young and middle aged glaucoma patients. It is unlikely, however, to solve the problems involved in treating elderly glaucoma patients who need assistance from other people.

Acknowledgement

The authors wish to thank AB Hassle, Mölndal, Sweden, for the supply of Ocusert®.

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TIMOLOL VERSUS PILOCARPINE SEPARATELY OR COMBINED WITH ACETAZOLAMIDE-EFFECT ON INTRAOCULAR PRESSURE

BY

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Fifty-eight patients with intraocular hypertension or primary open angle glaucoma participated in a double masked randomized study. Timolol in concentrations 0.25% and 0.5% was compared with 1% or 4% pilocarpine. Acetazolamide (250 mg \times 3) was added if intraocular pressure (IOP) was uncontrolled with the highest concentrations tested.

No statistical difference was found in hypotensive effect between pilocarpine and timolol neither on ocular hypertension nor glaucomas. The additive hypotensive effect of acetazolamide was the same for both substances. Once a day administration of timolol was sufficient in 17 of 90 cases controlled merely by topical administration.

Key words: timolol - pilocarpine - acetazolamide - once a day administration - additive effect - comparative study

Timolol maleate (Blocadren) a β -adrenoreceptor blocking substance was introduced by Hall et al (1970). Topical treatment with timolol was found to reduce intraocular pressure (IOP) both in healthy volunteers (Katz et al 1976) and in those with elevated IOP (Zimmerman & Kaufman 1977a,b). Several comparative studies have shown that timolol administered twice a day has equally high hypotensive effect as pilocarpine (Bischoff 1978, Boger et al 1978, Katz 1978, Flax et al 1978, Radius et al 1978, Airaksinen 1979, Diamond et al 1979, Schiffer 1979). However, according to Zimmerman & Kaufman (1977b) the effect of one single application of timolol persists more than 24 h. The aim of this paper was to study to what extent

potensive effect of timolol once a day was sufficient and comparable to pilocarpine administered four times a day. The other purpose of the study was to compare the additional effect of oral acetazolamide in cases uncontrolled by topical therapy.

Material

Eighty patients with primary open angle glaucoma or ocular hypertension were included for a double masked study (Table I). Forty patients were examined at the Central Hospital Huddinge and 18 patients at the Central Hospital in Skövde. Differences according to age, IOP or other parameters were found in the two groups which have therefore been pooled.

The criteria for admission to the study was an untreated IOP of > 22 mmHg by Goldmann applanation tonometry. The pressure is a mean value of two measurements separated by at least one h. The eye with the highest pressure determined the treatment for both eyes. Forty-one patients already receiving therapy for glaucoma or ocular hypertension were instructed to discontinue their previous treatment for one week. Nine patients had their washout period shortened since a rise of IOP was suspected to be harmful. In one case with a tendency to corneal oedema and IOP of 38 mmHg the therapy was already started on the third day. In six cases with glaucomatous changes and IOP above 30 mmHg (mean 34.5) the therapy was started on the fourth day and in another 4 cases (3 glaucomas, 1 ocular hypertension, mean IOP 32) therapy started on the fifth day.

The criteria for glaucoma were defined as glaucomatous visual field defects according to Goldmann perimetry and/or glaucomatous cupping of the optic disc with a C/D ratio of at least 0.8 or documented progress of the cupping. According to this definition 34 patients in the study were considered to have open angle glaucoma and the rest 24 were defined as ocular hypertension.

The drugs used were timolol maleate in concentrations of 0.25% and 0.5% or acetazolamide in concentrations of 1%, 2% and 4%. The substances were packed in

Table I

	Sex		Mean age (range)	Ocular hyper- tension	Glaucoma
Pilocarpine	17 M	11 F	68 (54-80)	10	18
Timolol	14 M	16 F	67 (51-84)	14	16

identical bottles marked Vial A, B and C respectively. For the timolol group this corresponded to 0.25% once a day, Vial B to 0.5% once a day and Vial C twice a day. For the pilocarpine group Vial A equalled 1%, Vial B 4% and Vial C 4% four times a day.

In a randomized fashion the patients entered the timolol or pilocarpine group and continued in their respective groups throughout the study. One of the investigators, uninformed of the actual therapy, interviewed and examined each patient while the other determined the therapy and informed the patient.

Thirty patients were treated with timolol and 28 with pilocarpine. The treatment started with Vial A and after one week the IOP was measured. If uncontrolled (< 22 mmHg) the treatment was changed to Vial B. After another week the IOP was recorded and if still uncontrolled Vial C was given. If IOP was not even controlled on Vial C concomitant oral therapy with acetazolamide 500 mg \times 3 was given. When a patient reached an IOP of less than 22 mmHg receiving Vial A, B or C in combination with acetazolamide he was maintained on this therapy and examined after 2 and 6 weeks. Before and during treatment visual acuity, applanation tonometry, slit lamp observation and funduscopy were performed and pupal visual fields, blood pressure and pulse were recorded.

The statistical analysis was based on the eyes with highest IOP and the Wilcoxon signed rank test was used.

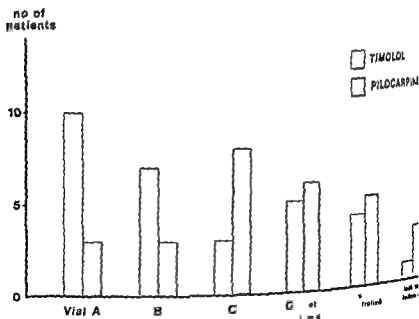


Fig. 1
Distribution of patients according to final treatment.

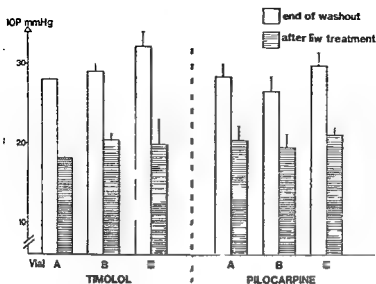


Fig 2

untreated IOP in mmHg \pm SEM and mean IOP after 6 weeks therapy in 34 patients (20 timolol 14 pilocarpine) regulated by topical treatment

Results

Distribution of patients according to final treatment is shown in Fig 1. Twenty patients were controlled with topical timolol of which 17 (9) with administration only once a day (the figures in brackets refer to the number of included angle glaucomas). Five (3) more patients were regulated with timolol and tolamide in combination. Four (3) patients remained uncontrolled and 1 (0) it was lost to follow up.

In the pilocarpine group 14 (10) patients were controlled with topical therapy, 6 (3) needed additional acetazolamide, while 5 (4) remained uncontrolled and 1 (1) were lost to follow up. There exists no statistical difference between the number of patients controlled on topical timolol and pilocarpine respectively and hypotensive effect is the same in both glaucomas and ocular hypertensions.

Fig 2 the untreated base line IOP and the IOP after 6 weeks maintenance therapy is shown. No statistical difference is found in base line pressure between the pilocarpine and the timolol group. The mean pressure decrease for topical timolol was Vial A 9.9 mmHg, Vial B 8.6 mmHg and Vial C 12.4 mmHg. Corresponding figures for pilocarpine were 7.0, 7.0 and 8.8 mmHg. Some patients (4 from the pilocarpine group and 3 from the timolol group) showed a tendency to therapy

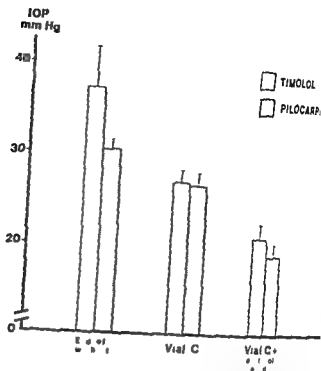


Fig 3

Mean untreated IOP in mmHg \pm SEM mean IOP with maximum topical treatment and mean IOP with concomitant acetazolamide in 11 patients (5 timolol 6 pilocarpine) regulated by topical treatment and additional acetazolamide

escape. They had an IOP < 21 mmHg at the end of the dose adjustment but after 11 weeks maintenance therapy the IOP was 24.0 and 23.4 in the group.

The additional IOP lowering effect of acetazolamide in patients not controlled by Vial C was equal for the timolol and the pilocarpine group (Fig 3).

In 3 persons (2 in the timolol and 1 in the pilocarpine group) a slight progress of glaucomatous visual field defects was noted.

Patients' complaints of adverse side effects varied both in frequency and type. The pilocarpine treated patients showed a higher incidence of complaints due to myopia and miosis. One timolol treated patient was observed with subconjunctival haemorrhage. However none of the adverse effects were severe nor did they cause discontinuation of the therapy.

The pulse rate showed a minimal but significant reduction from 71.2 ± 2.6 in timolol treated patients while systemic blood pressure was not influenced.

Discussion

study comparing pilocarpine and timolol in various concentrations timolol d to be at least as effective as pilocarpine in reducing an elevated IOP 67% in timolol group and 56% in the pilocarpine group were controlled on topical treatment alone. Similar results have previously been reported by other authors (off 1978, Boger et al 1978, Plane et al 1978, Radius et al 1978, Airaksinen, Schiffer 1979).

According to Zimmerman & Kaufman (1977b) the IOP reducing capacity persists for 24 h. The long lasting effect of timolol was also demonstrated in this study. 17 patients were controlled on once a day regime. 10 of them on the lowest concentration (0.25%). The increase of therapy to twice a day administration only treated another 3 patients. Several authors (Moss et al 1978, Plane et al 1978, Bag et al 1979) have also used the 0.25% concentration but administered it twice a day. It is remarkable that 50% of the IOPs controlled by topical timolol were treated on 0.25% once a day. Maybe for some patients a reduction of their timolol dose would not increase their IOP but diminish the risk of drug side effects. Furthermore, a once a day regime is the most convenient therapy for the patient.

The additive hypotensive effect of acetazolamide to timolol has been described by others (1978), Bischoff (1978) and Keates (1979). In this study the additive pressure lowering effect was equivalent in both the timolol and pilocarpine group. This is a little astonishing as acetazolamide decreases the production of aqueous humor (Friedenwald 1949, Becker 1954) a mechanism which is also attributed to timolol (Coakes & Brubaker 1978, Yablonski et al 1978, Dausch et al 1979). Can reduced aqueous in flow interfere detrimentally with the normal physiology of the cornea and trabecular meshwork? Long term investigation may be necessary to evaluate this.

Timolol like other β -adrenergic blocking agents affects systemic blood pressure and cardiac regulation. According to Alvan et al (1980) only a small amount of timolol (0.5 ng/ml) is found in the plasma during topical treatment with 0.5% twice a day. However, this amount seems to be sufficient to effect cardiac regulations as a significant decrease in pulse rate was observed in this study. Whether this dose is sufficient to interfere with the nutrition of the optic nerve is not known. The effect on blood pressure was noted. Neither were any adverse systemic reactions reported by Britman (1979) or Jones & Ekberg (1979) noticed by us. In two timolol and one pilocarpine treated patients regulated at the controls some decrease in preexisting visual field defects was noted. No certain conclusions can be drawn as the follow up time is rather short but long term studies are in progress.

Acknowledgement

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THE EFFECT OF MELPERON ON OCULAR READAPTATION TIME AS ASSESSED BY A NEW RECORDING TECHNIQUE

BY

H. BERGMAN¹, S. BORG¹, H. HÖGMAN², H. LARSSON and B. TENGROTT¹

A new simplified technique recording readaptation time after photostress (RAT) is described. The psychometric properties in terms of interobserver and retest reliability were tested, and the effect on RAT after intake of melperon at two different dose levels was investigated and correlated to blood plasma levels. The results show that there was a satisfactory constancy of RAT at each occasion but stability over a 1 month period could not be demonstrated. Significant dose dependent changes were recorded after intake of melperon but the prolongation of RAT was not significantly correlated to blood plasma levels.

Key words: nystagmus, central nervous system, drug effects.

Registration of readaptation time following a brief exposure to glare has been shown to be a sensitive method of registering the depressive effects of alcohol, oxazepam respectively on the central nervous system of man (Högmán et al., Bergman et al., 1979).

In the present study the psychometric properties in terms of interobserver and retest reliability of a new simplified recording technique for RAT was investigated (Experiment I). In Experiment II the effect on RAT of melperon (Buronil[®]) at different dose levels was investigated and correlated to plasma levels of melperon.

Method of Recording Readaptation Time

Readaptation time (RAT) was determined by registering the pause in optokinetic nystagmus (OKN) that appears after photo stress on the human eye. The method for RAT registration has been presented elsewhere (Tengroth et al 1977).

The apparatus used in this experiment is a modification of the earlier version. A schematic of the apparatus is shown in Fig. 1. The subject is placed in front of a white painted spherical screen with a diameter of 50 cm. The flash lamp and the OKN stimulus projector are placed above the subject's head. The flash lamp is an ordinary photo flash (Sunpak GX 24 colour type 6500 K, pulse time 0.7 ms and pulse energy approx. 25Ws). The light flash (covering 180° of the visual field) is reduced 100 times to the luminance of 10^4 cdm^{-2} . It is directed from the screen and glares the subject. The OKN stimulus is generated by a lamp driven by a rotating drum with black and white stripes. The stimulus (covering a horizontal area of 20° and a vertical area of 10° of the visual field) is projected on the screen. To facilitate accommodation, the subject wears glasses with lenses of +4 dioptres. The horizontal movement of the stimulus can be varied from 0 to 360°/sec. The optimum velocity of useful optokinetic nystagmus varies from subject to subject but is in the neighbour-

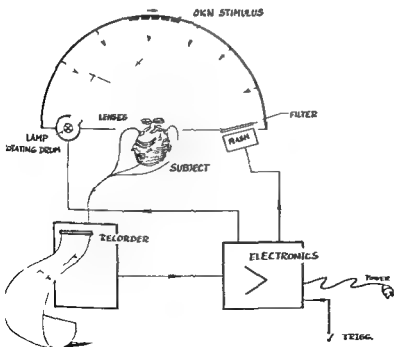


Fig. 1

The modified equipment for determination of readaptation time (RAT)

hood of 10 /sec. The luminance of the OKN stimulus is 10^{-6} cdm² (scotopic level). Eye movements are transduced by EOG electrodes to an electrical signal of about 1 mV, which is recorded by a Siemens Mingograph. The signal is amplified and via the clock unit is used to trigger the flash lamp to make it flash only when the subject's eyes are directed to the target ahead. The electronics also control the stimulus direction and velocity, the level of the signal and the flash.

The subject is dark adapted during 5 min before measuring the readaptation time. When this adaptation has taken place 5 RAT registrations are made at one minute intervals.

In the further analysis of the data, the mean of the five measurements is calculated.

Experiment I

Subjects and statistical analysis

Thirty healthy volunteer undergraduate psychology students (24 women, 6 men) served as subjects. Their mean age was 29.6 years (SD 8.7). None of them had taken drugs for at least two weeks prior to the test sessions. Each subject was investigated twice, at the same time of day, at an interval of one month.

To investigate the repeatability of the RAT registrations, the consistency of individual's RAT on one and the same day as well as the test-retest stability over a longer time period were assessed. Thus, the internal consistency reliability for both occasions was estimated by calculating Cronbach's Alpha coefficient (Cronbach 1951). High intercorrelations between registrations result in high coefficients. The retest stability for each series was estimated by calculating the product-moment correlations between results at the two occasions. Further, changes in mean RAT between occasions were tested for significance by Student's *t* test for correlated samples.

Results

Table I presents retest and internal consistency reliabilities of RAT. The Cronbach's Alpha coefficients were 0.96 and 0.89, respectively, for each occasion, and

Table I
Product-moment correlations between occasions, mean values (\bar{x}), standard deviations (SD), *T* test for correlated samples (*T*₉₉) and alpha internal consistency coefficients

Rat	\bar{x}	SD	<i>T</i> ₉₉	Alpha
First occasion (I)	-0.2	1.5	4.8	0.96
Second occasion (II)	1.1	3.2		0.89

1 degree of consistency in the registrations for each separate occasion
ver there was no stability over the one month period the product moment
ation between the two occasions was -0.05 ns Furthermore there was
-tistically significant change in the group means over the 1 month interval
) 42 df 29 $P > 0.05$)

Experiment II

cts and statistical analysis

healthy male subjects all medical personell of the institutions mean age 29
(sd 2.8) volunteered None of them had consumed drugs for a period of four
prior the investigation and smoking was not allowed during the experiment
eron was taken per os fasting RAT was recorded at 8 a.m. and then
diately after intake of respectively 25 and 50 mg melperon per os and
ved 60 180 300 and 420 min thereafter

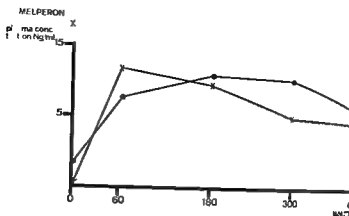
nous blood samples were drawn 60 180 300 and 420 min after drug intake
entration of melperon in plasma was estimated by gas liquid chromatography
(son 1976)

ie RAT changes at different points of time after the intake of 25 mg and 50
respectively were tested for significance in a two-way analysis of variance
n with time after intake as a repeated measures factor Dose level was the
een groups factor A trend analysis using orthogonal polynomials was also
formed (Winer 1971) In order to obtain the nearest possible equal interval time
the following RAT values were included in the analysis RAT II RAT 60
180 RAT 300 and RAT 420 In order to assess the relationship between level
melperon in plasma and RAT the product moment correlation between mean
ma level and mean RAT responses for the RAT 60 RAT 180 RAT 300 and
420 was calculated

alts

changes in RAT at the above mentioned five points of time after intake of 25
and 50 mg melperon (Buronal®) per os are shown in Fig. 2 and the results of the
way analysis of variance in Table II There was a statistically significant
erence in RAT prolongation between the two dose levels ($P < 0.05$) Further
e there was a statistically significant change of RAT regardless of dose level (P
01) mainly due to the quadratic component of the trend over time ($P < 0.001$)
ximum RAT prolongation for the 25 mg group was recorded 3 h after intake
in the 50 mg group 5 h after intake but there was no significant over all

(Fig. a)



(Fig. b)

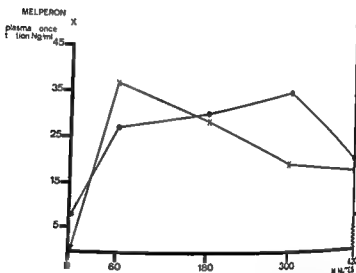


Fig. 2

Prolongation of RAT and plasma concentration at different times after intake of 25 (Fig. a) and 50 mg (Fig. b) per os mean values

interaction between dose levels and change over time ($P > 0.05$). The baseline (0) was not reached during the 7 h period. Changes of drug concentration in plasma are shown in Fig. 2 for 25 mg and 50 mg respectively. For both doses the maximum concentration was recorded 1 h after intake of melperon. The product moment correlation between the curves of plasma concentration recordings based on 5 points of time after drug intake for the two dose levels was 0.51 and 0.72 respectively.

Table II

Two-way repeated measures polynomial analysis of variance of RAT at five times after intake of 25 mg and 50 mg melperon per os. Source of variation, degrees of freedom (df), mean squares (MS) and F test.

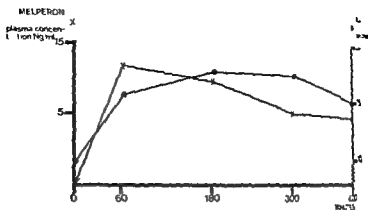
Source	df	MS	F
<i>Between subjects</i>			
Dose level (D)	1	1695.4	5.44
Error	10	311.3	
<i>Within subjects</i>			
Times (T)	4	85.1	4.59 *
D × T	4	23.9	1.29
Error	40	18.5	
T (linear)	1	72.2	3.07
D × T (linear)	1	21.9	> 1.00
Error	10	23.5	
T (quadratic)	1	255.7	21.40* *
D × T (quadratic)	1	59.4	5.00*
Error	10	11.9	
T (cubic)	1	6.7	> 1.00
D × T (cubic)	1	0.1	> 1.00
Error	10	20.8	

$P < 0.05$ * $P < 0.01$ * * $P < 0.001$

Discussion

The first experiment showed that the new equipment used to record RAT had satisfactory psychometric properties in terms of consistency of measurement at one occasion. However, there was no test-retest stability. The lack of stability over time limits the use of RAT registration when making intra-individual comparisons. Significant changes in RAT were recorded after intake of melperon. The prolongation after 50 mg was of the same magnitude as after intake of 20 mg oxazepam per os (Bergman et al 1979). Furthermore, the RAT prolongation appeared to be dose-correlated. In a similar experiment, intake of 25 mg and 50 mg melperon per os was found to significantly influence critical flicker fusion in a dose-dependent manner (Molander & Duvholt 1976). Furthermore, the maximum effect on critical flicker fusion was recorded after approx. 3 h (ibid). The maximum effect of melperon in this study was found to occur after 3 h and the effect could still be

(Fig. 1)



(Fig. 1b)

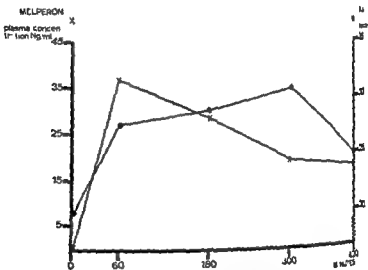


Fig. 2

Prolongation of RAT and plasma concentration at different times after intake of 20 (Fig. a) and 50 mg (Fig. b) per os mean values.

interaction between dose levels and change over time ($P > 0.05$). The base line (0) was not reached during the 7 h period. Changes of drug concentration in plasma are shown in Fig. 2 for 20 mg and 50 mg respectively. For both doses the maximum concentration was recorded 1 h after intake of melperon. The product moment correlation between the curves of plasma concentration and recordings based on 5 points of time after drug intake for the two doses were 0.51 and 0.72 respectively.

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COMPUTER DENSITOMETRY OF RETINAL NERVE FIBRE ATROPHY

A pilot study

BY

MATS LUNDSTRÖM and JAN-OLOF EKLUNDH

Loss of nerve fibre layer opacity and a granular appearance of the retina are important funduscopic signs in optic atrophy. In this pilot study serially obtained fundus photographs from a case with developing optic atrophy were subjected to computer densitometry. A monotonic increase in local density variations was found during evolution of optic atrophy. The change in density variations closely followed the funduscopic change in nerve fibre layer opacity. This method makes it possible to detect and quantify diffuse atrophy of the retinal nerve fibre layer.

Key words: optic atrophy, ophthalmoscopy, fundus photography, image processing, densitometry.

Funduscopic defects of the retinal nerve fibre layer have been reported in several cases (see Lundström 1977, a recent review). Such defects may be focal or diffuse. Focal defects are often prominent due to the abrupt change in colour at the border between atrophic and normal retina. Diffuse atrophy, on the other hand, is stable and slowly advancing, is difficult to recognize. Lundström & Frisén (1979) described the dynamic evolution of nerve fibre atrophy in a case with amaurotic lesions of the anterior visual pathway. Two major types of changes were seen during the evolution of atrophy: a successive decrease in the nerve fibre layer opacity and a successive loss of the striated pattern. The decrease of nerve fibre layer opacity and the simultaneous increase of retinal mottling appeared in black and white fundus photographs as an increase of local grey level variations.

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Grey level variations in black and white negatives can be recorded by densitometry as density variations. This report describes a method capable of recording and quantifying density variations in black and white negative film. The results presented here are obtained from fundus photographs of the nasal retina of the eye.

Materials and Methods

In an attempted suicide a 35 years old man received a bullet injury to the right eye near the knee of the optic chiasm. There was an immediate and permanent visual loss in the right eye and an upper temporal field defect in the left eye. The earliest signs of peripapillary nerve fibre atrophy in the blind right eye were followed by ophthalmoscopy and fundus photography. Full clinical and technical details are given by Lundström & Frisén (1975). From the series of black and white negatives one set of negatives representing different stages of atrophy were selected for computer evaluation. Only negatives showing the optic disc and the nasal peripapillary retina were chosen because they showed the best uniformity in sharpness and centering. The sharpest negative from days 25, 30, 33, 40 and 45 after the injury were chosen for study. The negatives were scanned by a Ferranti film scanner in a 512×768 raster corresponding to a linear resolution of about 40 lines/mm. The effective number of grey levels was about 40. Because the nerve fibres are radially orientated around the optic disc the matrix was sampled along circles concentric with the disc. In this way density values were recorded along circles in a digital picture built up by a linear raster. In order to keep the sampling error low a first order linear approximation was used. Resolution along a circle thus remained at about 21 lines/mm.

The measure of the density variations in the negatives was computed as follows:

1. The centre of the optic disc was determined in interaction with the operator. From each negative circular sweeps along arcs in the nasal peripapillary retina were selected. The radii of the circles ranged between 2.5 and 4.5 optic disc radii.

2. Each arc was represented by a series of values $\{x_1, x_2, \dots, x_n\}$ corresponding to the density level along the arc. From this series of values the corresponding series of absolute differences between adjacent density values along the arc $\{y_1, y_2, \dots, y_{n-1}\} = \{x_1 - x_2, x_2 - x_3, \dots, x_{n-1} - x_n\}$ was computed. This series represents the amplitudes in the density curve.

3. Picture elements overlying major vessels were discarded by excluding the largest 5% to 15% of the elements in this way.

4. The arithmetic mean of the remaining y_i (mean amplitude) was computed for each arc and used as a measure (M) of the local density differences.

Results and Discussion

les of original photographs and their digital transforms recreated on film by printing device are given in Fig 1. Examples of the density variation along the arc are shown in Fig 2.

The mean amplitude (M) of each arc was computed. The values from corresponding arcs in the series of negatives representing different stages of atrophy were compared. Because the actual values were of little importance we have ranked the amplitudes for each arc. The ranking list is shown in Table 1. As can be seen in Table 1 there was a monotonic change in density variation through the series of negatives. Ophthalmoscopy during the same period of time revealed a successive decrease in nerve fibre layer opacity. This decrease in retinal opacity corresponded to an increase in local grey level variations in the black and white negatives. The observed monotonic increase in density variations in the same series of negatives indicates that densitometry of fundus photographs may be used to detect changes in nerve fibre layer appearance.

In this pilot study picture processing and densitometry was performed with a computer technique. This expensive method was used in order to make the analysis easier. In the digitization process a scanner with a 40 μm aperture was used and the effect of film granularity could therefore be neglected. The diameter of the nerve fibre bundles in our negatives was estimated to be 90-240 μm . Therefore the linear resolution of the scanner was considered sufficient to record the striated pattern. However in the series of negatives there was an increasing sharpness of the vessel edges due to the increase in atrophy. This change together with the decrease in opacity made a negative to-negative comparison of density components corresponding to the striated pattern difficult. Further away from the optic disc the striated pattern was not as prominent as in other parts of the peripapillary retina. By excluding five to 15% of the largest density variations the effect of retinal vessels and striations was diminished and changes in striation and opacity were enhanced. The actual value chosen in the interval was arbitrary. Being radially distributed the nerve fibre bundles were examined on concentric circles with the optic disc. Circles with a radius of 2.5 to 4.0 times the radius of the optic disc were used. When a larger radius was used the characteristic striations disappeared. This agrees with the ophthalmoscopic finding of decreased visibility of the nerve fibre layer at this distance from the optic disc. Conversely, close to the optic disc abundant reflexes from the internal limiting membrane and local variations in background pigmentation impaired definition of the nerve fibre layer. The effect of other structures such as retinal blood vessels and choroidal vessels were eliminated by excluding the largest density variations. The computer quantitated the more extended variations in contrast between different negatives.

B



A

Fig 1

Pictures from day 25 (A) and 47 (C) respectively and their corresponding day 25 pictures (B+D). The matrix was sampled along arcs concentric with the optic disc shown in the pictures (B+D)

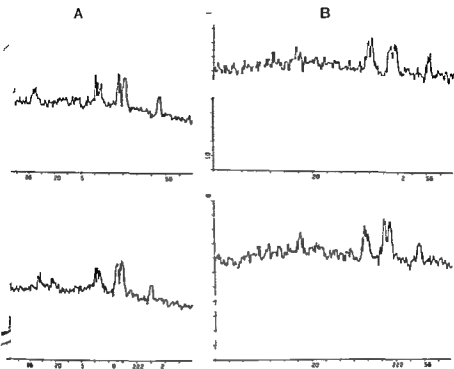


Fig. 2

trimey curves from the pictures shown in Fig. 1. The curves represent density values along two arcs on day 25 (A) and the same arcs on day 47 (B).

Table I

in° of the negatives. The negatives are identified by the number of days passed after the exposure. The radius is expressed as a multiple of the radius of the disc. Lowest ranking number (1) indicates the lowest mean amplitude.

negative (days)	Radius														
	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	2	2	2	2	3	2	3	2	2	2	2	2	2	2	2
33	4	3	3	3	2	3	2	3	2	3	3	3	3	3	3
40	3	2	2	4	4		4	4	2	4	4	3	4		4
4		4	4	2	2	4		2	4	2	4	2	2	4	2

caused by exposure variations. The sharpness of the negatives on the other hand is a point because in blurred negatives the grey level variations decrease and thus also the density variations. Therefore a set of slightly blurred negatives were also examined. Still a monotonic increase in density variations was found and was more pronounced than when sharp negatives were examined. The electroretinogram is extremely difficult to reproduce in photographs taken at different times. According to Behrendt & Doyle (1962) differences of centering on the retina may cause errors up to 10% in image size. Moreover centering of the eye is not exact. Considering the series of negatives the increasing anisopticity could not be established from one circle alone per picture due to geometrical errors. Six circles were however sufficient.

In this case of traumatic optic atrophy the changes in the retinal nerve fibres were due to a more or less simultaneous degeneration of all retinal nerve fibres. Therefore these findings can not be directly transferred to studies on a progressive loss of single nerve fibres or bundles of nerve fibres as in glaucoma. This pilot study shows however that it is possible to detect and quantify the atrophy of the retinal nerve fibre layer. Similar findings in atrophy of perimacular bundles have been reported by Tagami (1979).

Acknowledgments

The authors wish to express their gratitude to professor Björn Tengdahl for general advice.

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A DEFECT IN THE BLOOD RETINA BARRIER IN THE OPTIC NERVE HEAD REGION IN THE RABBIT AND THE MONKEY

BY

TOR FLAGE

The permeability properties of the tissues in the optic nerve head region have been investigated in monkey and rabbit using horseradish peroxidase as an histochemical tracer. Following intravenous administration the tracer diffused from the peripapillary choroid into the different parts of the optic nerve head region and also into the receptor layer of the sensory retina adjacent to the innermost layer of the tissue. This observation demonstrates a defect in the blood retina barrier in this region. The defect in the permeability barrier is most likely located either to the retinal pigment epithelium near the optic nerve head to the innermost layer of tissue or to the junction between these tissues.

Key words: blood retina barrier defect rabbit monkey horseradish peroxidase optic nerve head ultrastructure

Blood retina barriers play an important role in the present concept of retinal biology. It is generally accepted that the endothelium of the retinal vessels and retinal pigment epithelium (RPE) form the anatomical basis for the barriers. Cells in these tissue layers are interconnected by tight junctions. Peyman & Bok (1979) demonstrated the blood retina barriers in the primate retina using horseradish peroxidase as a protein tracer. In experiments using this tracer we studied permeability properties of the tissues in the optic nerve head region in rabbit monkey and found that horseradish peroxidase diffused from the peripapillary choroid into the optic nerve tissue proper thus demonstrating a defect in the

blood optic nerve barrier (Fløge 1977). In the same study the tracer was observed in the peripapillary sensory retina indicating a defect in the barrier in this region. In the present article this observation will be given greater detail.

Material and Methods

The material and the procedures have previously been described (Fløge 1975, 1977). From 15 rabbits and 4 monkeys (*Cercopithecus aethiops*) were examined in the eye. Horseradish peroxidase (HRP) Sigma type II molecular weight approximately 40,000 daltons molecular diameter of about 4.5 nm was used as an histochemical tracer. It was administered by intravenous injection. The amount of tracer used for the rabbit was 100 mg/kg body weight. For the monkeys the amount varied between 35 to 40 mg/kg body weight. The time from the injection of the tracer until the enucleated eye was immersed in a cooled fixative called the exposure time to HRP ranged from 4.5 to 10 min for the rabbit. The same period in the monkeys varied between 20 and 60 min. The eyes were run by light microscopy either in 5 to 10 μ m sections obtained on a freezing microtome or in thick sections either unstained or following staining with toluidine blue. The eyes were also examined in a Siemens Elmiskop 1A unstained or stained with uranyl acetate and alkaline lead.

Results

Following intravenous injection HRP leaked out of the choroidal capillaries into the peripapillary choroid the tracer spread into the tissues of the optic nerve region of rabbit and monkey in a distinct pattern. The details of this pattern have been described elsewhere (Fløge 1975, 1977). Bruch membrane the border of Elschnig and small islands of connective tissue in the inner retina (KIT) were heavily stained by the tracer.

The most striking observation in the present context was the finding of HRP in the peripapillary sensory retina (Fig. 1). This finding was also present in the eyes initially fixated by perfusion. In the rabbit eyes with exposure time to HRP less than 10 min the tracer was not regularly present in the peripapillary retina. In the monkey eyes the staining caused by the tracer in the retina was generally less than in the rabbit eyes.

In the sensory retina HRP was located in the subretinal space and between the outer and inner segments of the visual cells. No staining was present in the inner retinal layers. The HRP stained peripapillary sensory retina and subretinal



Fig 1

nerve head region in rabbit following intravenous injection of horseradish peroxidase. Black staining marks the presence of the tracer. Small arrows point to the tracer in the papillary sensory retina. No staining is present in the inner retinal layers except in the subretinal connective tissue. Long arrow points to subretinal space. The border of Elschnig (E) is heavily stained by the tracer which has also spread into the adjacent optic nerve tissue (ON). $\times 300$

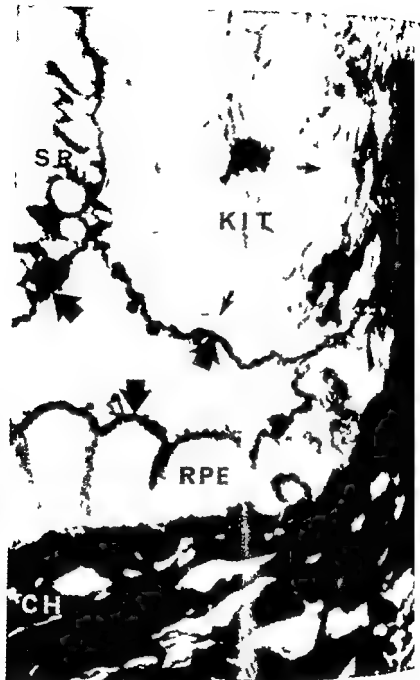


Fig. 2

Part of Fig. 1 in higher magnification showing the relationship between the choriocapillary border tissue of Elschnig (E) the retinal pigment epithelium (RPE) Kuhn's interstitial tissue (KIT) and sensory retina (SR). Small arrows point to tracer in the intercellular space of KIT. Broad arrows point to tracer lining the subretinal space. The area at the bottom left is CH (choriocapillary endothelium).



Fig 3

on micrograph monkey optic nerve head region. Broad arrows point to Bruch membrane and connective tissue in Kuhnt intermediary tissue (KIT) stained by horseradish peroxidase. Open arrows mark the tracer between the glial cells of KIT. Arched arrows point to the tracer in the subretinal space. Optic nerve (ON). Retinal pigment epithelium (RPE). Inner segment of visual cell (IS). L-ranyl and lead $\times 4280$.



Fig. 2

Part of Fig. 1 in higher magnification showing the relationship between the basement membrane of Elschnig (E), the retinal pigment epithelium (RPE), the subretinal space (SR) and inner limiting membrane (ILM). Small arrows point to tracer in the SR. Broad arrows point to tracer in the subretinal space. The arrowhead indicates a junction between the last RPE cell and a glial cell (X 9000).

defect in the blood-retina barrier is most likely located either in the RPE near the optic nerve head or to the junction between these tissues. These possible routes of diffusion will be the subject of further investigation (Ringvold 1980).

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EVIDENCE THAT HYPOTONY IN RETINAL DETACHMENT IS DUE TO SUBRETINAL JUXTAPAPILLARY FLUID DRAINAGE

BY

AMUND RINGVOLD

One case of aphakic retinal detachment with increased intraocular pressure is described. Overnight the intraocular pressure fell abruptly to normal values and simultaneously the detachment had advanced to the optic disc border. The hypothesis is formulated that decreased intraocular pressure in eyes with retinal detachment is brought about because intraocular fluid is leaking out of the eye through a subretinal route to the peripapillary tissue. Additional support for this posterior aqueous outflow is obtained from clinical studies showing that maximum hypotony is reached when the detachment has advanced to the optic disc border.

Key words: retinal detachment - intraocular pressure - subretinal fluid - glaucoma

Since Graefe in 1869 (see Leber 1916) considered the intraocular pressure to be normal or lower in eyes with retinal detachment, this observation has been confirmed both before and after the introduction of tonometry (see Doherty 1967). The search for the cause of this IOP decrease has also given rise to many reports with conclusions roughly falling into two groups. Some authors report that there must be an increased outflow in such eyes, perhaps because of subretinal absorption by the choriocapillaris (Leber 1916, Kleiner 1933), whereas other investigations lend support to the view that the IOP is low because aqueous production is reduced in eyes with detached retina (Becker 1963, Dobbie 1963, Evans & Regan 1963, Regan & Rousseau 1966, Svardalen 1970).

The present study is an attempt to analyze aqueous humour dynamics in eyes with retinal detachment. On account of observations in one case of:

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ment and a clinical material of retinal detachment the hypothesis is put forward that the IOP decrease in eyes with retinal detachment may be due to apillary drainage of subretinal fluid.

Case Report

A 60-year-old myopic woman had successful intracapsular cataract extraction on the right eye resulting in visual acuity of 20/20 (+3 sph). Eight months later she underwent cataract extraction on her left eye with postoperative visual acuity of 20/40 (+2 sph). During the last operation there was considerable vitreous protrusion after lens extraction and although no vitreous loss occurred, corneal suturation was impeded by vitreous substance in the anterior chamber.

Three weeks after cataract extraction on the left eye the patient came to the hospital with a history of visual loss on this eye and periodic frontal headache on the same side. The examination revealed that the right eye was unchanged from the description above. The IOP was 19 mm Hg by applanation tonometry and this eye remained normotensive during the following observation period.

In the left eye which also showed normal aphakic anterior chamber depth the ruptured iris face was perforated by some thin vitreous threads protruding into the anterior chamber. Gonioscopy revealed some goniosynechiae in the upper nasal quadrant and although the peripheral coloboma a vitreous connection was attached to the corneal scar. The retina otherwise appeared regular. A low retinal detachment with two pre-equatorial breaks in the temporal half was present involving the macula and covering the 4-12 o'clock area. The superior limit reached about one disc diameter from the optic nerve (Fig. 1a). The visual acuity was finger counting at 3 m and IOP was 4 mm Hg. Despite 500 mg acetazolamide per os and drop of timolol maleate (Blocadren 0.5%) in the left eye the IOP was still 37 mm Hg the

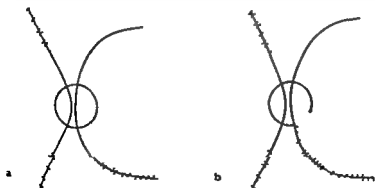


Fig. 1

Schematic drawing of the left fundus with the retinal detachment (dotted area) and its position to the optic disc: a) as IOP was 42 mm Hg and b) as IOP was extremely low (not measurable) next morning. Note the detachment reaches the disc border in the 4-6 o'clock region in Fig. b.

later. The next morning however the anterior chamber was deep the IOP was extreme hypotony giving a zero value by applanation tonometry and the anti-glaucoma drugs were promptly stopped. At this time a striking change was observed in the peripheral area as the detachment now had advanced to the disc border in the 4-6 o'clock area a marked tunnel shaped retinal elevation (Fig 1b). Otherwise the fundus appeared as evening before. The eye was operated with encircling band, local haemangioma cryopexy and drainage of subretinal fluid. The next morning the IOP was 10 mm Hg.

Material and Methods

In order to determine the relationship between the detachment distance and the size of the detachment in conventional rhegmatogenous detachments schematic fundus drawings of such patients admitted to the Eye Department Rikshospitalet in 1976 were reviewed. The drawings had been made by the skilled ophthalmologists after clinical examination with indirect ophthalmoscopy and with a three mirror Goldmann lens. All eyes included in the material had primary retinal detachment and there had been no previous surgery for ophthalmic disease. A total of 128 detachments were used. In those cases where the detachment did not touch the disc border the shortest distance (called a) between the posterior detachment border and the optic disc was measured. Subsequent detachments were cut out with a pair of scissors weighed and the percentage detached area was then calculated in % comparing its weight to the weight of the fundus area.

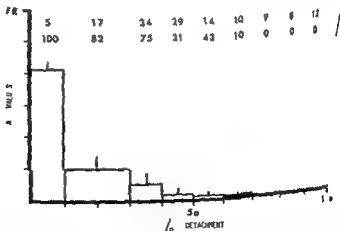


Fig 2

The curve shows the relationship between detached area in % of total fundus on the abscissa and its corresponding distance a to the optic disc along the ordinate. a = distance between detachment and optic disc. FR = fundus radius of the fundus. a = distance representing the greatest possible a value. Number of cases in each group indicated in the upper line whereas the lower line shows in % how many cases in respective groups that did not reach the disc.

Results

relationship between the distance a and the size of the detached area is seen in

Discussion

The occurrence of acute hypotony combined with deep anterior chamber in eyes with retinal detachment similar to the present case was first described by Schnabel (1927) and later it has been observed by several authors (see Beigelman 1929). As summarized by the last one, different mechanisms have been made responsible for this phenomenon. However, the coincidence of an abrupt fall in IOP as the detachment reached the disc through the tunnel shaped extension indicates that hypotony in this case was due to leakage of subretinal fluid along the optic nerve. This interpretation is supported by the fact that the IOP was again raised from immeasurable values in the pre-operative phase to 10 mm on the first day after closure of retinal breaks with the buckling procedure.

Compared to the conventional rhegmatogenous retinal detachment with moderate hypotony in the pre-operative stage, the referred case was unusual in showing raised IOP on arrival at the hospital. This was probably due to changes in the vitreous meshwork secondary to cataract extraction and accordingly it is suggested that there was nothing unusual with the detachment itself. The syndrome of acute hypotony may therefore represent an exaggerated state of the frequently occurring moderate hypotony in eyes with retinal detachment.

The second part of this study was performed to test whether the hypotony in eyes with conventional retinal detachment may be brought about because intraocular fluid seeps through the retinal breaks leaving the eye through a subretinal route to connective tissue around the optic nerve as visualized in Fig. 3. This flow would meet substantial resistance first in that part of the subretinal space where the retina is attached to the pigment epithelium, i.e. along the route defined as the shortest distance (a) between the detachment and the disc. Accordingly, the degree of hypotony would be inversely proportional to the distance a . It should be stressed that when a is zero and the detachment thus has reached the disc, the IOP would not decrease any more even if the detachment continues to increase. Consequently, maximum hypotony should be achieved at the moment the detachment reaches the optic disc. To test the validity of the hypothesis it seems therefore essential to answer the following two questions: 1) How large is the detachment when it just reaches the optic disc? 2) At what detachment size is maximum hypotony reached? As to the first question, Fig. 2 shows that roughly 40-70% is detached as it reaches the optic disc.

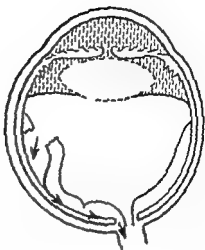


Fig 3

Arrows indicate the suggested posterior outflow pathway of the intraocular fluid in the subretinal space to the juxtapapillary tissue in eyes with retinal detachment.

In order to answer the second question data from the Syrdalen (1941) (1933) and Dobbie (1963) investigations are adapted to graphs by the author in Figs 4 and 5 respectively. Because the degree of hypoxia is influenced by the age of the detachment (Syrdalen 1970) Fig 4 presents data based on the total material and one graph with data derived from the 1-16 detachments. It is seen in Figs 4 and 5 that maximum hypoxia is present in the range from below 50% to 66% detachment which is the same interval where the size of detachments are falling when they just reach the disc (comp. Fig. 1). This coincidence speaks in favour of the presented hypothesis.

Interestingly a subretinal defect in the blood retina barrier has been demonstrated next to the optic nerve (Flage 1980) and more directly this opening has been demonstrated in *in vitro* experiments by showing free diffusion of horseradish peroxidase into the connective tissue surrounding the optic nerve (Flage & Ringvold 1981).

As stated above according to the hypothesis the degree of hypoxia is inversely proportional to the distance a . However since the interface between the external limiting membrane and the pigment epithelium is not completely impermeable (Berman 1969) it seems reasonable that this substance is changed or even to some extent washed out through the flow. In other words the flow is increasing when the distance a becomes shorter there should be a decreasing subretinal flow resistance per length unit along the distance a .

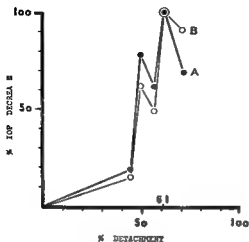


Fig. 4

obtained after statistical calculations of the material presented by Svardalen (1960 p. 10). The five points along the abscissa are found by calculating mean values from those detachment numbers lying closely together. Curve A includes the total material as mean of the following groups (44.04-44.33) (49.06-49.33) (55.34-55.94) (60.5-60.94-61.0) (69.16-70.2-70.9-74.86). Curve B represents numbers from the first 14 days only (44.33) (49.06-49.33) (55.34-55.94) (60.94-61.0) (69.76). The corresponding mean differences are seen along the ordinate. In both curves the greatest IOP difference was set to 100%.

It is suggested to be one reason why small detachments do not alter the tension significantly as pointed out by Dobbie (1963) and seen in Figs 4 and 5.

In the case that the hypotony in retinal detachment is due to subretinal or vitreous fluid drainage, tonography seems unsuitable to examine the aqueous humor dynamics in such eyes because the extrabulbar counterpressure induced at the cornea through the weight of the tonometer is likely to impair this posterior flow.

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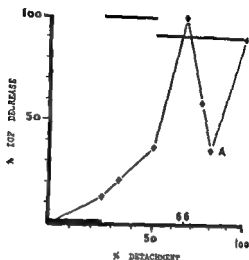


Fig 5

Curve A represents the material presented by Kleiner (1933 p 49) and Regan based on mean values of the respective groups of % detachment and their corresponding mean IOP differences. Dobbie's material (1963 p 160-161) shows IOP values for detachments smaller than 20% larger than 50% and the region in between. The indicated as thick lines. In both investigations the greatest IOP difference was found

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THE ERG c WAVE IN VITELLIRUPTIVE MACULAR DEGENERATION (VMD)

BY

SVEN ERIK G NILSSON and KLAS OLAV SKOOG

Dark ERG registrations and EOG recordings were obtained from six patients with vitelliruptive macular degeneration (VMD). In all cases the EOG was highly pathological but the a - and b -waves of the ERG were normal. This is typical of VMD which starts as a generalized defect of the pigment epithelium. Four patients showed no evidence of a c -wave. The other patients demonstrated small c -waves but only under certain stimulus conditions. Thus varying stimulus durations, intensities and frequencies are sometimes needed to decide whether or not ERG c -waves can be elicited in different diseases or suspected disorders. The findings are in agreement with the presence of a generalized pigment epithelial defect in VMD since the major positive component of the c -wave is generated by the pigment epithelium receptor complex.

Keywords: electroretinography, clinical method, c -wave, retina, pigment epithelium, vitelliruptive macular degeneration.

The hereditary condition originally described by Best (1905) has since been referred to with a large number of designations. We have chosen the term vitelliruptive macular degeneration (VMD) (Krikl et al 1966). This condition has been studied extensively both clinically and electrophysiologically. Very thorough reviews may be found elsewhere (Deutman 1971, Krikl 1977). A Swedish pedigree of VMD was described by Barkman (1961), Nordstrom et al (1972) and others. As it appears from the highly pathological EOG (Krikl et al 1966, Francois 1968, Deutman 1969) in the presence of normal a - and b -waves of the ERG (Francois et al 1963, Krikl et al 1966, Francois 1968) VMD starts as a generalized defect in the pigment epithelium. Structural evidence to this effect is not complete but two cases

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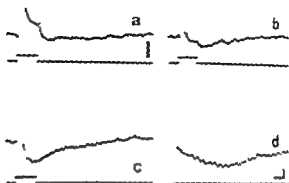


Fig. 2

Registrations from a patient (S. D.) who lacked c waves under all circumstances. Stimulus intensity 3.5-4 = 5.5 and 4.0 rel. log units in Figs. 2a, b, 2c and d, respectively. Amplitude calibration 100 μ V (the calibration line in Fig. 2a also applies to Fig. 2c). Stimulus duration 1 s in Figs. 2a, c (indicated on lower lines). Stimulus duration 1 s in Fig. 2b. Time calibration 0.5 s in Fig. 2d.

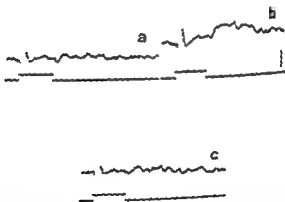


Fig. 3

Registrations from a patient (H. D.) who developed a c wave when stimulation of the c wave amplitude were elicited through repeated stimulations and registrations. Stimulus intensity 4.0 rel. log units. Amplitude calibration 100 μ V. Stimulus duration 1 s (indicated on lower line). Fig. 3a from the beginning of the series (average of four recordings). Fig. 3b from min. 6-7 (average of four recordings) and Fig. 3c from min. 10-11 (average of four recordings).

of 1 s flashes the trough became very deep and did not reach the iso-electric level after the end of the stimulus (Fig. 2 d)

of the patients (H D and S D) were studied with repeated stimulations and flashes with 1 s flashes at log ret. intens. 4.5 for 20 min. One of them (S D the patient as in Fig. 2) showed a shallow trough (as in Fig. 2 b) throughout the

The second patient (H D) in which no c wave was seen at the beginning of the test (Fig. 3a) demonstrated a small but quite clear c wave after 3-4 min. It was normal in amplitude (approximately 120 μ V) at 6-7 min (Fig. 3b) and from 10 min disappeared again (Fig. 3c).

At the two lowest intensities the so-called d.c. potential was seen, lasting until the end of the stimulus (Figs. 1 and 2a). It appeared to be normal.

In conclusion all six patients lacked normal c waves. Four of them (J D, S D, A D and B S) showed no c wave at all. One patient (G D) displayed small c waves only at low intensities. Another subject (H D) also showed small c waves but upon repeated registrations which served to provoke c wave amplitude changes.

Discussion

Although there are a few healthy individuals who lack c waves (Taumer 1970; von Koenig & Skoog unpublished data) the large majority of subjects have clear c waves. When going from log ret. stimulus intensity 3.0 to 3.5 Skoog & Nilsson (1964) obtained a steady increase in c wave amplitude.

The small number of patients in the present study does not permit formal statistics. However, given the scarcity with which the authors and others have encountered normal individuals without c waves, it is highly unlikely that the successive appearance of six VMD patients with no or in two cases atypical c waves is a coincidence. The conclusion seems to be that VMD patients, at least those belonging to the Swedish pedigree, have no or pathological c waves.

In fact our present results in subjects with a diffusely pathological pigment epithelium as reflected by abnormal EOG and histology (see introduction) were expected. Such a condition would most likely lead to a decreased P I (slow major negative component of the c wave originating in the pigment epithelium receptor complex) and an intact slow P III (slow negative component of the c wave from the Müller cell receptor complex) (see introduction). The total absence of P I would lead to a deep negative trough (isolated slow P III) instead of a c wave (Noell 1953 and others). Some P I activity would give shallow troughs, straight lines or low c waves. If hypothetically in a few persons with healthy eyes the slow P III was very large one would encounter a low or even absent c wave. This is a possible explanation as to why some normal persons seem to lack a c wave.

Oscillations of the standing potential (SP) of the eye and amplitude of the c wave occur in a parallel way in man (Nilsson & Skoog 1973). The presence of a dark trough and light peak in the EOG reflecting the oscillation of the SP changes in illumination is not equivalent to the presence of a c wave. Central retinal artery occlusion can abolish both oscillations without affecting the average c wave amplitude (Textorius et al 1978). Thus the delay in itself does not necessarily lead to an anticipation of small c waves.

It is particularly interesting to note that one patient (H D) with a fast time after the fast ERG-potentials probably reflecting a poor P I function, despite a reasonable c wave about 4-10 min after the onset of registrations corresponding roughly in time to the development of the first maximum in the c wave amplitude oscillations in normal subjects (Skoog & Nilsson 1974b). In this patient we showed some oscillatory ability since the Arden index was 1.70. The SP and c wave oscillations are related and thus it was not wholly unexpected that, by provoking the oscillations, small but clear c waves could be brought about for some time in patient (S D) with no evidence of a c wave in spite of the provocation with 10 registrations/min had a shallow trough to begin with and an Arden index of 1.4. It cannot be decided on the basis of this study whether the cyclic change is due to P I in P III or in some other slow component which may take part in the build-up of the c wave.

Concerning the patient (G D) with c waves after weak stimuli only it is tempting to suggest that the condition reflects a failure of the diseased pigment epithelium to produce more than a small positive P I even if the stimulus became strong enough to give rise to a large negative slow P III from the inner intact retina. The large slow P III would completely bury the small P I.

In conclusion patients with vitelliruptive macular degeneration (AMD) may have no or very small c waves because of a pigment epithelial dysfunction. As pointed out in this small study varying stimulus conditions must be used in order to test whether or not c waves are present in different diseases or suspected diseases. One should try to elicit oscillations of the c wave amplitude by repeated stimulation with 10 registrations. In this way otherwise hidden c waves may sometimes be found. As pointed out by Textorius et al (1978) one cannot perform reliable c wave tests without taking into account the effect of their amplitude oscillations.

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CORRELATION OF VISUAL EVOKED POTENTIALS OPHTHALMOLOGICAL AND NEUROLOGICAL FINDINGS AFTER UNILATERAL OPTIC NEURITIS

BY

H BYNKE, I ROSEN and M SANDBERG WOLLHEIM

Forty-two patients were tested with pattern reversal visual evoked potentials (VEPs) 1-8 years after an episode of acute unilateral optic neuritis. The stimulation was produced by dot as well as checker-board pattern. An unexpectedly large proportion (12 cases) showed normal VEP latencies from the affected eye. In these patients the initial visual acuity was on the average significantly better than in cases with prolonged VEP latencies. They also had significantly fewer remaining ocular signs than the patients with abnormal VEPs. On the other hand, no correlation was found between the VEP findings and the incidence of CSF abnormalities, multiple sclerosis and HLA types. In 14 patients of which 13 belonged to the group above, repeated VEP tests were performed from the acute onset of the disease and up to 2 years afterwards confirming that a considerable normalization of VEP latencies occurs.

Key words: optic neuritis - visual evoked potentials (VEP) - pattern reversal stimulation - ocular signs - CSF abnormalities - multiple sclerosis - HLA types

Previous studies of visual evoked potentials (VEPs) after optic neuritis normal results of pattern reversal VEP have been reported very rarely e.g. in 3-6% of normals of Halliday et al. (1972 and 1973a) and Shahrokh et al. (1978). Also in patients with suspected multiple sclerosis and a history of optic neuritis normal latencies have been found in few cases (Halliday et al. 1973b, Asselman et al.

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1975, Matthews et al. 1977, Shahrokhi et al. 1978). The ultimate visual acuity was completely uncorrelated with the VEP latency but it was clearly correlated with VEP amplitude (Halliday et al. 1973a). Therefore the latency shift of VEPs is to be established at the time of the acute optic neuritis and did not appear to be influenced by the recovery processes which lead to an increase of the amplitude.

In this hospital all patients with acute optic neuritis have been regularly followed for years by regular neurological and ophthalmological check-ups. Cerebrospinal fluid abnormalities and HLA types have also been determined (Lund, Wollheim 1975, Sandberg-Wollheim et al. 1975). Preliminary VEP results from some of these patients revealed an unexpectedly large proportion of patients with normal or almost normal VEP latencies. In order to penetrate this matter further an unselected group with varying degrees of remaining ocular defects after optic neuritis was tested with VEP. The results were correlated with the clinical symptoms and signs, frequency of remaining defects and number of co-existing signs of multiple sclerosis.

As the present study proceeded the authors had the opportunity of including a group of new cases of optic neuritis with repeated VEP tests from the onset of the disease and up to two years afterwards. The purpose of this study was firstly to confirm that patients with symptoms of acute optic neuritis exhibit characteristic VEP abnormalities when tested and measured with the techniques and secondly to follow the time course of normalization.

A preliminary account of the results has been published (Rosen et al. 1979).

Material

The material consisted of 42 patients, 18 males and 24 females who were followed up for observation after an episode of acute unilateral optic neuritis. Their ages at the time of the final examination ranged between 23 and 51 years (mean 35 years). The observation time from the onset of the disease to the final check-up ranged from 0.5 to 8 years (mean 4.3).

At the time of the acute episode the majority of the patients suffered from retrobulbar pain and all of them had reduced vision in the affected eye. The visual acuity was 0.9–0.6 in 13 cases, 0.5–0.2 in 12 cases and 0.1 or less in 17 cases. All patients exhibited defective colour vision and central visual field defects. The fundus of the affected eye was normal (retrobulbar neuritis) in 34 patients and abnormal (papillitis) in 8. In one single case there was amblyopia due to an atrophic diseased eye.

Methods

Pattern stimulation was produced by an electronically controlled stimulator comprising a matrix of 64 light-emitting diodes (response time 90 ns, stimulus frequency 1 Hz); half were lit at a time and switched to provide pattern reversal (Evans et al. 1974). Two sized screens were used. One provided a matrix of dots and the other a checker board.

The electronic control device and hence the timing of reversal was identical for the two sets of stimuli. The patients were placed 1.5 m in front of the screen at which distance luminance was 11 008 lux. The luminance of the dots of the dot screen was 640 cd/m² (the screen area). The luminance of the squares of the checker board screen was 400 cd/m² (50% of the screen area). The visual angle for each of the screens was 3.3° and between adjacent dots/squares 2.5°. The diodes of both screens emitted a yellow light. The test was performed in a light room with the patient's gaze fixed at the upper edge of the screen. Our experience is in agreement with that of others in that selective stimulation of the lower field causes minimal variability of the response (Halliday 1972; Lehmann & Mir 1976). A single channel bipolar lead (C - O) was used. The signal was amplified by a regular EEG amplifier (0.5-70 Hz) and 2 × 100 traces for each stimulus and eye were averaged in a Didac averaging computer and photographed for documentation.

Latency of the dominant positive (positive at neck) deflection P100 was determined as the amplitude from this peak to the following negative peak of the response. This mode was chosen because it was sometimes difficult to define a negative peak preceding P100. This was never the case with the negativity following P100.

In 149 patients pattern reversal VEP tests from both eyes were made at least once, i.e. at the end of the final check up. In 13 patients repeated VEP tests were performed during the observation time from the initial acute phase of the disease and up to two years afterwards. In one patient was examined with repeated VEP tests from the acute phase of the disease (Fig. 2). As the observation time was only 4 months he was not included in the rest of the study. Altogether 149 pattern reversal VEP tests were made bilaterally.

Table 1

Values for the normal control group (29 eyes in 14 subjects, age 23-59 years (mean 33.6 years))

	P100 latency (ms)		Amplitude (μV)		Side-difference			
					(ms)		(μV)	
	MI	SD	MI	SD	MI	SD	MI	SD
Pattern reversal								
dots	89.5	9.6	5.6	2.3	0.2	0.8	0.7	0.7
Pattern reversal								
checker board	107.5	7.0	3.9	2.3	4.0	5.3	0.7	0.6

For reference identical VEP determinations were made on a series of 11 normal subjects whose age distribution was similar to that of the patients (23-59 years, mean 37.5 years). Repeated recording of VEP gave variations of P100 latency of less than 5 ms. The constancy of the P100 latency at repeated tests is also demonstrated in the clinically unaffected eye in the present clinical material (Fig. 9B).

Clinical eye examinations

Repeated routine ocular examinations were made in all 49 patients to check up a detailed neuro-ophthalmological examination was performed in 22 cases by one of us, assisted by a well trained ophthalmic technician. On this occasion the visual acuity was tested with Monoyer's 20-table and the colour vision with Bostrom Kugelberg's pseudo-isochromatic plates. The visual fields were examined with Goldmann's kinetic perimeter. Several white test objects were used. Particular care was taken to map the central field and to compare the results of both eyes as regards the position and shape of the isopters. Swinging flash light of high and low intensity was used to test the pupillary light response. The fundus included examination of the retinal nerve fibre layer with intense red-free light.

Neurological examinations

All patients underwent a neurological examination and a lumbar puncture at the onset of ocular symptoms. None of the patients had had previous neurological symptoms including optic neuritis and a thorough examination was performed in each case. In the CSF a pleocytosis was found in 58% and in 40% two or more bands in the gamma globulin fraction on agar gel electrophoresis were demonstrated. In patients with MS an oligoclonal gamma globulin pattern is the common CSF abnormality (Laterre et al. 1970).

The HLA type of each patient was determined earlier (Sandberg-Woller 1975) by serotyping for 23 different HLA antigens and by NLC typing. In the present patient material 11 patients carried HLA A3, 19 patients HLA B7 and 18 patients were DR2 positive.

During the observation period each patient was re-examined at least once in order to establish whether or not the patient had developed symptoms of MS. The last neurological examination was undertaken on the same day as the final ophthalmological examination and the VEP-test. Altogether 42 patients developed signs of lesions in other parts of the CNS during observation time and were considered to suffer from MS.

Statistical methods

Student's *t* test was used for comparison of independent sets of measurements and chi-square for comparison of frequencies.

The final evaluation was made blindly, i.e. none of the examiners knew the results of the other two.

Results

ference to the results of the VEP tests of the 14 normal subjects (Table I) the patients were grouped into three different categories according to the latencies (Table II A). Group I consisted of 12 patients with completely normal VEPs, i.e. a normal latency time, a latency prolongation of the response from the eye as compared with the unaffected eye of less than 3 ms (mean of dot and board values). Group II comprised 21 cases with a latency prolongation of more than 3 ms from the affected eye of more than 3 ms (range 3–70.5 ms). Only 5 of the 21 had latency differences between the eyes of less than 10 ms. Group III consisted of 9 patients showing P100 latencies above the normal range from both

eyes. The initial mean visual acuity of the affected eye was 0.50 (SD 0.39) in group I and 0.7 (SD 0.29) in group II. It had thus been significantly better in the group of patients with normal VEPs than in the group with abnormal VEPs ($P < 0.05$). The occurrence of other initial ocular symptoms and signs were found to have been distributed in both groups.

A correlation was found between VEP latencies and the ultimate visual acuity, even when other remaining ocular signs were taken into account, i.e. defective vision, visual field defects, reduced afferent pupillary light response and atrophy. Significant differences existed between the groups. Thus the patients with normal VEPs exhibited significantly fewer remaining ocular signs than those with abnormal VEPs ($P < 0.005$). Table III.

Table II A
Subdivision of patients from VEP criteria

No	Criteria	Age		Observation time (years)	
		Range	Mean	Range	Mean
12	Normal VEP Latency prolongation on affected side less than 3ms	20–4	31.9	0–8	3
21	Pathological VEP from affected eye Latency prolonged on more than 3 ms	20–50	33.4	1	3.5
9	Pathological VEP from both eyes	28–51	33.9	1.5	5.1

Table II B
VEI values for the three subgroups of patients

		P100 latency (ms)		Amplitude (μ V)		Side-difference			
						(ms)		(μ V)	
		M	SD	M	SD	M	SD	M	SD
Affected eye	I	87.8	5.2	4.4	2.4	-0.9	3.7	0.19	1.0
	II	108.0	20.6	3.8	2.0	17.9	17.0	1.20	1.2
	III	134.3	11	3.6	1.1	12.0	19.8	1.1	1.5
Control eye	I	92.5	9.8	4.7	1.9	1.0	2.8	-0.3	1.4
	II	120.9	23.1	4.3	3.0	22.6	20.1	1.7	1.8
	III	193.8	19.3	4.4	2.2	97.3	19.7	0.5	1.6
Unaffected eye	I	87.7	5.0	4.1	2.2				
	II	103.1	24.4	3.0	2.1				
	III	122.3	13.2	4.7	1.1				
Total	I	88	9.8	4.4	2.4				
	II	108	20.6	3.8	2.0				
	III	134	11	3.6	1.1				

Table III
Clinical ophthalmological findings at the time of the final check up

	Entire group	I	II	III
Reduced visual acuity	1/39 (38%)	4/11 (36%) $P > 0.05$	9/0 (45%)	9/8 (25%)
Defective colour vision	11/39 (28%)	0/11 (0%) $P < 0.05$	9/20 (45%)	2/8 (25%)
Visual field defect	16/39 (41%)	9/11 (81%) $P < 0.05$	11/20 (55%)	3/8 (38%)
Reduced afferent pupillary light response	16/39 (41%)	2/11 (18%) $P < 0.02$	12/20 (60%)	2/8 (25%)
Optic atrophy	23/39 (59%)	4/11 (36%) $P < 0.01$	17/20 (85%)	4/8 (50%)
Σ Pathological signs	$21\% \pm 1.7\%$	1.09 ± 1.13 $P < 0.005$	9.90 ± 1.59	1.63 ± 1.69

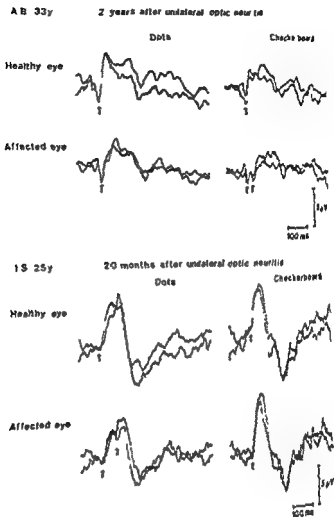


Fig 1

Pattern reversal visual evoked potentials recorded from two different patients at the final check up. For details of stimulation and recording see Methods. Visual calibrations are given in the figure. The patient A B showed a slightly defective a paracentral visual field defect and a pale optic disc in the affected eye. The showed a slight reduction of visual acuity in the affected eye but no other retinal. The visual acuity at the time of the acute optic neuritis was 0.9 for A B and 0.1. P100 latency during the initial phase was 167 ms for A B and 170 ms for I S. I S showed a pleocytosis of CSF and I S a pathological agar gel-electrophoresis.

The three groups of patients did not differ in terms of frequency of initial normal CSF findings or distribution of HLA types A3, B7 and DW2. Altogether the 42 patients developed signs of lesions in other parts of the CNS during the observation time and were considered to suffer from multiple sclerosis. Three of them belonged to group I, i.e. those with normal VEPs and one case to group III, i.e. those with bilateral VEP abnormalities.

For the normal controls as well as the clinical material the P100 latency was systematically longer with checker board stimulation than with dot stimulation (Tables I and II). The average difference calculated for each of the normal eyes was 13.0 ms (SD 9.2 ms). A similar difference was seen for the eyes affected by optic neuritis (average difference 10.0 ms) as well as the fellow eyes (8.1 ms).

The difference of latency between the affected and fellow eyes was about the same for dot and checker board stimulation (average difference with dot stimulation 11.6 ms as compared with 13.9 ms for checker board stimulation). In the group of 21 patients with pathological latency prolongation from the affected eye, 10 showed a larger latency difference with checker board stimulation and 11 a larger difference with dot stimulation. In one case the latency difference was exactly the same with the two types of stimulation.

As regards the shape of VEP, clearcut differences were found in individual cases for the two types of stimulation. Examples are given in Fig. 1. In case A-B, dot stimulation produced symmetrical responses but checker board stimulation evoked a bi-phasic P100 response from the affected eye. In case I-S, dot stimulation produced a somewhat lower amplitude response with a notch from the affected eye, the response to checker board stimulation being almost identical.

For the 14 cases followed by repeated VEP tests from the initial phase of the disease and up to two years afterwards, the P100 latencies for the affected and fellow eyes were plotted in Fig. 2A and B. The course of the VEP latency shifts varied individually but the three groups of patients, i.e. those with normalized P100s (Group I), with remaining latency asymmetry (Group II) and with bilateral latency prolongation (Group III) could be identified. Group II was further divided into Group IIA with entirely unaffected VEP latency on the clinically affected eye and Group IIB with an initial slight latency prolongation also for the clinically unaffected eye. A similar initial latency prolongation on the unaffected eye was seen for one patient in Group I (Fig. 2B). For the affected eyes a rapid as well as a more prolonged phase of recovery of latency over several months could be distinguished. In most cases the amplitude underwent a faster recovery than did the latency, usually reaching a peak after 2-4 months. Then there was a general tendency of the amplitudes to return to a somewhat lower level than for the clinically healthy eye.

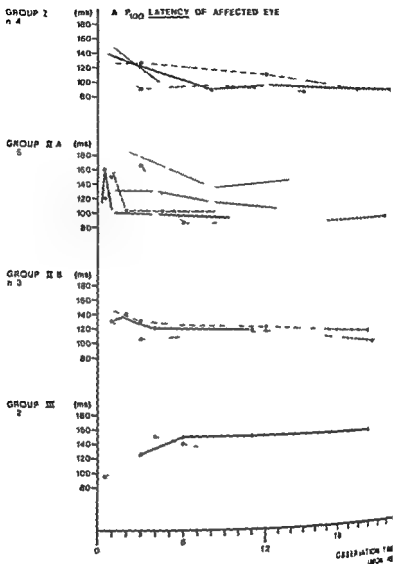


Fig 2 A

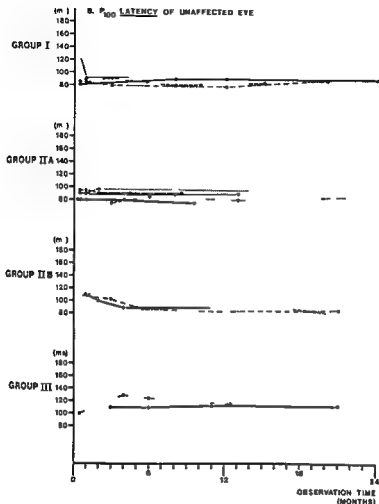


Fig 2 B

Fig 2 A and B

Longitudinal follow up of 14 cases of acute optic neuritis. A: P₁₀₀ latency of affected eye. B: P₁₀₀ latency of unaffected eye. Groups I, II, and III: patients fulfilling the criteria of VEP responses at the time of final check up according to the classification given in Table II. A: Group IIA: patients with remaining VEP asymmetry and with no significant change of latency from the fellow eye. Group IIB: patients with remaining latency asymmetry and with an initial latency prolongation also from the clinically unaffected eye.

Discussion

Twelve of our 42 patients (29%) with a previous episode of acute optic neuritis had a normal pattern reversal VEP. This frequency is much higher than in the series of Halliday et al (1972, 1973a) and Shahrokh et al (1978).

Our stimulating technique differed from that used by these authors in a lower luminosity (15 cd/m² as compared with 110 cd/m² for the checkerboard squares) and a smaller screen (33° as compared with 81–99°). However, we cannot explain the high number of normalized VEP latencies in our series. According to Nilsson (1978) a low intensity pattern reversal stimulus with light emitting diodes from a 3.9° screen turned out to be more sensitive in detecting abnormal responses than a high intensity checkerboard stimulus from a large screen (1000 cd/m² 8.8°). The method of selective stimulation of the lower half of the visual field does not seem to decrease the proportion of abnormal responses in patients with multiple sclerosis (Lehmann & Mir 1976). Furthermore, although influencing the absolute values of amplitudes and P100 latencies the method of selective stimulating, recording and measuring the VEP responses can hardly detect small relative changes on the affected eye at repeated examinations of the same individual.

One explanation of the results obtained may be that our material included a greater number of cases with mild optic neuritis. The observation that the visual acuity of the affected eye was significantly better and the fact that there were fewer remaining ocular defects in the group with normal VEPs than in the group with abnormal VEPs give some support to this hypothesis.

Another explanation of the divergent results may be that in previous series the optic neuritis was often a sign of generalized MS. It is not known to what extent the remaining latency prolongation of VEP is a sign of disseminated demyelination or a remnant of the acute episode of optic neuritis. Patients with the sporadic form of MS often develop bilateral latency prolongation of VEP without any history of optic neuritis (Bynke et al 1977). Only a small proportion of patients with acute optic neuritis develop signs of generalized MS (Sandberg Wollheim 1979, Bynke et al 1979).

The incidence of other initial ocular symptoms and signs and of CSF abnormalities was evenly distributed among the patients, making it very unlikely that patients with normal VEPs had another type of disease than those with abnormal VEPs.

Furthermore, the longitudinal study with repeated VEPs during the follow-up phase supports the assumption that the initial disease was an organic disease of the optic nerve.

In agreement with the results of Halliday et al (1973a) no correlation was found

een the VEP latency prolongation and the final visual acuity. However a relation was found between the VEP latency and other remaining ocular signs. A sophisticated test battery including more detailed examinations of the central field, more subtle tests for colour vision and differences between eyes of evoked latency (Rushton 1975, Galvin et al 1976, Slagvold 1978) would probably have demonstrated even more clearcut correlations between VEP latency and clinical findings after optic neuritis.

The study shows that a normal and symmetrical pattern reversal VEP test months years after an acute episode of unilateral eye symptoms does not in any way exclude the possibility that this episode was due to optic neuritis.

The VEP values after acute optic neuritis from our experience do not give conclusive information as to the risk of developing multiple sclerosis. Three of four cases of multiple sclerosis occurred among the patients who had normal VEP values after the episode of the optic neuritis.

We found a bilateral VEP latency prolongation after unilateral optic neuritis in 11 cases (Group III). Amblyopia is known to produce not only amplitude changes but also latency prolongation either from the amblyopic eye or from the fellow eye (Møller & Nilsson 1978). In only one of the nine cases the diseased eye was also amblyopic. In the other eight cases a reasonable explanation of the VEP latency prolongation from the fellow eye is a subclinical optic neuritis of that eye. In one of these cases the latency prolongation of the contralateral eye observed to develop during the follow up period. Moreover among the 14 cases followed during the follow up phase at least four showed an initial latency prolongation from the fellow eye demonstrating that this eye was not entirely unaffected (Fig. 2).

There was a systematic difference of latency between dot and checker board stimulation in the present study both among normal subjects and among patients with optic neuritis despite identical timing of the stimulus and identical size of the stimulus. The response amplitude produced by the two types of stimuli was similar (average value of 5.6 μ V for dot stimulation as compared with 5.9 μ V for checker board among normal controls). The two types of stimuli differ in form and luminosity (dots: circles 3 mm diam. 640 cd/m²; checker board: squares 1 cm² 45 cd/m²). The earlier latencies for dot stimuli cannot be attributed to the difference of luminosity only because Nilsson (1978) found shorter latencies for dot stimulation than for checker board stimulation of considerably higher luminosity. The probability may be considered that partly different groups of nerve fibres or cortical neurones were involved in the two types of test. The investigation does not indicate that any of the two types of stimulation is especially sensitive in revealing remaining conduction abnormalities after demyelination. In practice we have found it of value to apply both types of stimuli when using VEP as tests of disseminated CNS demyelination.

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APPLICATION OF TWIN FLASH STIMULI FOR SCOTOMATOPIC MACULAR DISEASES ISOLATION OF LOCAL RESPONSES BY TWIN FLASHES

BY

MARIKO MITSUYU YOSHIHITO HONDA and AKIRA NEGI

Double flash ERG was examined for patients with visual loss from macular and optic nerve disorders and the responses were compared. h values ($B_1/B_2 \times 100$) of the former were larger than normal values when the interstimulus intervals were between 200 and 500 mseconds and abnormality of h was seemed to correspond to the functional impairment of macula. On the other hand h values of the patients with optic nerve diseases remained in the normal range. These facts indicate that double flash ERG is a very useful method to estimate the cone function clinically and can be employed as a routine examination for patients with suspected cone dysfunction.

Key words: double flash (twin flash) — stimulus intensity — stimulus interval h ($= (B_1/B_2)/(B_1 \times 100)$) — macular degeneration

In our previous works double flash ERG was studied on rod-dominated, cone-dominated and mixed retinas of mammals and its usefulness in separating cone function was emphasized (Mitsuyu 1978a, b). This time the double flash technique was applied to patients with visual loss from macular and optic nerve disorders and difference of responses was investigated.

Materials and Methods

Nine normal volunteers (aged between 20–30) and seven patients were examined. The tested eye was previously fully mydriated with phenylephrine hydrochloride (0.5%) and tropicamide (0.5%) drops. Benoxinate was dropped on the cornea. A corneal contact lens electrode of Kawabata's type was attached as usual.

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le Reference electrode was attached on the earlobe. The electric signal was led by pre amplifier (time constant 0.3 seconds) and photographed. Diffuse light was delivered by a xenon flashtube which was designed to discharge a (twin) flash of the same energy in the range from 0.3 joule to 2.0 joule. The interval between the first and second flash within a pair could be freely changed. In investigation the interval remained between 50 mseconds and 1000 mseconds. 10 min in the dark, double flash stimuli were applied continuously. When amplitudes of ERGs became constant, stimulus intervals of the double flash were varied from 50 mseconds to 1000 mseconds and the responses were compared. Stimulus frequency of the double flash was always 0.1 Hz and stimuli of several intensities were employed. The amplitudes of the ERGs by the first and the second flash were measured from the bottom of the a wave to the top of the b-wave as indicated in Fig. 1 and were named B_1 and B_2 . The index $(B_2 - B_1)/B_1 \times 100$ (named k) was calculated for every pairs.

Results

Responses from normal controls. The results of normal controls is shown in Fig. 2. In this experiment, stimulus intensities of 0.3, 0.6 and 2.0 joule were used. Fig. 3 shows the k s obtained from normal controls when the stimuli of 0.3 joule were used. Responses from nine subjects were averaged. When the stimulus interval was shorter than 100 mseconds, the second ERG barely appeared and k was 100. When the stimulus interval became longer than 100 mseconds, the ERG appeared. With prolongation of inter stimulus intervals, the B_2 increased and accordingly k decreased. At an interval of 400 mseconds, an inflexion is observed in the curve. Then the second ERG increased rapidly with an increase in the inter stimulus intervals and reached almost the same amplitude of B_1 at 700 mseconds. Fig. 4 k s of 2.0 joule stimuli are shown. In this case, a measurable ERG was induced by the second flash when the stimulus intervals was as short as 100 mseconds, on the descending shoulder of the first b-wave. When the stimulus



Fig. 1

B_1 and B_2 were measured as indicated in this figure

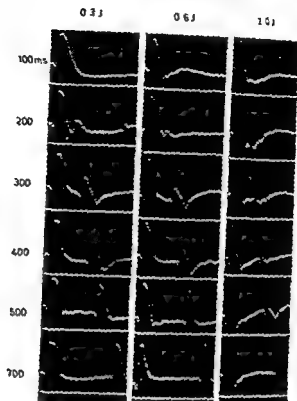


Fig 2

Double flash ERG of a normal control. Stimulus intensities of 0.3 joule or less were employed. Stimulus intervals were between 100 milliseconds and 700 milliseconds. Calibration 50 microvolt.

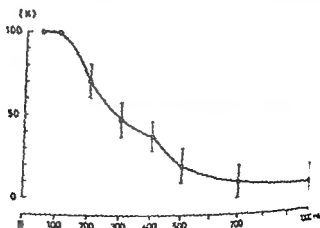


Fig 3

h values ($= (B_1 - B_2) / B_1 \times 100$) of normal controls when the stimulus interval was varied. Numbers along the ordinate represent h values and those along the abscissa represent stimulus interval. Nine experimental values were averaged and are shown.

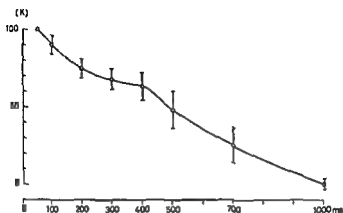


Fig 4

controls when the stimulus intensity was 2.0 joule. Nine experimental values were averaged.

was shorter than 400 mseconds. B₂ amplitude did not increase as rapidly as intensity of 0.3 joule. Accordingly, K of 2.0 joule decreased slowly and a flexion was made at 400 mseconds. K decreased more rapidly for the 2.0 intervals.

Application of double flash ERG for patients with macular diseases

Five patients were examined. One of the results (case 5) is shown in Fig 5. K values obtained from these five patients are indicated in Table I.

(Case 5)

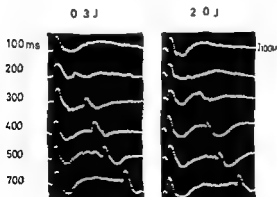


Fig 5

Double flash ERG of the case 5, right eye

Table I

K values of normal controls and those of case 1. For the case 1, 2 degrees indicated. Normal values are given by averaging of nine experiments.

Intensity	Interval mseconds	Normal SD	Case 1		Case 2		Case 3		Case 4	
			R	L	R	L	R	L	R	L
0.6 J	100	100 (8)	100	100	100	90	100	110		
	200	71 (10)	71	77	90	71	75	50	75	44
	300	48 (10)	59	82	10	30	57	20	60	40
	400	38 (9)			56	30	46	40	60	40
	500	20 (11)	25	57	61	36	75	70		
	700	10 (10)	0	20			0	75		
2.0 J	100	90 (6)	95	100	100	95	100	115		
	200	75 (6)	94	85	100	83	9	83		
	300	67 (7)	82	89	90	11	11	88		
	400	63 (9)			18	61	31	9		
	500	48 (12)	56	64	67	61	67	10		
	700	25 (12)	14	24			44	25		

(Case 1) Thirty year-old male. His visual acuity was 0.1 (n.c.) for the right eye and 0.1 (n.c.) for the left eye. By Ishihara & Farnworth Panel D-15 hue test, he was diagnosed as totally colour blind. His macula was degenerated and a small white reticular surrounding area of the macula was grayish clouded and black bone-corpuscles were seen in the retina. When the fundus was very carefully examined, some green spots were seen in this area. Fluorescein fundus angiography (Fig. 6) revealed indicating fundus flavimaculatus. His single flash scotopic ERG was within normal. But as is shown in Table I, most values of h_s in the double flash ERG of the present stimulus intervals of 200 mseconds and 300 mseconds were larger than normal.

(Case 2) Twenty year-old male came to the Kyoto University Hospital with a progressing visual loss. His visual acuity was 0.01 (n.c.) for the right eye and 0.1 (n.c.) for the left eye. Colour vision was tested by Ishihara & Farnworth Panel D-15 and he was diagnosed as totally colour blind, his left eye being normal. By funduscopy, some changes were seen for the right eye and a slight change was also seen for the left eye. Electroretinogram was flat for both eyes, showing widespread pigment epithelial dysfunction. h_s of the present stimulus intervals were above the normal range for both 0.5 joule and 2.0 joule stimulus intensities.

(Case 3) Twenty three year-old male was an elder brother of the case 2. He had no complaint but his left fundus showed a definite macular change very similar to the case 2. His visual acuity was slightly decreased (0.8 n.c.). Right eye was normal. Electroretinogram did not show light rise and pigment epithelial dysfunction to exist. h_s were within normal range for the stimulus intensity of 0.5 joule. But at stimulus intensity was 2.0 joule, h_s were above normal at the stimulus intervals of 200 mseconds and 300 mseconds for the left eye and at 300 mseconds for the right eye.

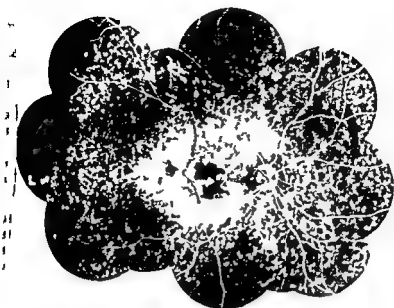


Fig 6

Fluorescein fundus angiography of the case 1 (right eye)

1) Nineteen year-old female had severe visual loss for several years. She complained of photophobia and could not see well under bright light. Her visual acuity was 0.1 (n.c.) for both eyes. She was totally colour blind by Ishihara & Farnsworth Panel D 15 blue test. Her visual evoked potentials were nearly normal on ophthalmoscopy and fluorescein fundus angiography except that a central reflex was seen. She was diagnosed as progressive cone dysfunction syndrome type 1. She was examined using double flash ERG. At 0.3 Jules stimulus intensity, Ks were considered to be above the normal range.

2) Twenty four year-old female complained of visual loss in the right eye which had occurred several months before. Her visual acuity was 0.1 (n.c.) for the right eye and 1.0 for the left eye. Funduscopy revealed a severe degenerative change with black pigmentation at the right eye. A slight change was also seen in the macula area of the left eye. Figure 6 shows the double flash ERG of the right eye of this patient. Ks were normal when stimulus intensity was 0.3 Jules was examined. Ks were above normal between the stimulus intervals of 100 mds and 400 mseconds.

Double flash ERG in the patients with visual loss caused by optic nerve disorders. All patients were examined.

3) Thirty-three year-old male complained of visual loss in the right eye for a month, and in the left eye for several years. His visual acuity was 0.2 (n.c.) for the right eye, and 0.1 (n.c.) for the left eye. On ophthalmoscopy the papilla of the right eye was almost

Table II
h values of case 6 and 7

Intensity mseconds	(Case 6)		(Case 7)			
	0.5 J		0.5 J		0 J	
	R	L	R	L	R	L
100	100	100	100	100	1	41
200	63	63	100	100	4	6
300	29	41	100	100	14	19
400	31	31	36	36	4	21
500	13	1	7	19	0	10
700	0	0	14	7	1	11

normal but that of the left eye was a little pale at the temporal margin. Table II shows values of this patient. All values are within or slightly below the normal range. It is found to be suffering from multiple sclerosis.

(Case 7) Forty year-old male had optic nerve atrophy and his visual acuity was 1/10 in both eyes. As is shown in Table II, his were within or slightly below the normal range.

Discussion

Double flash ERG has been examined for human subjects by some workers (Burian & Spivey 1959, Elenius 1964, Algvere & Westberg 1967, Fiala & Rouck 1962) and Elenius (1969) studied it on patients with cone dysfunction.

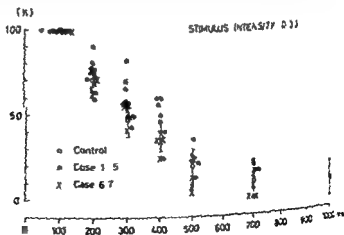


Fig. 7

h values of the case 1-7 are plotted with control values. Stimulus intensity is in mJ.

ter showed that in two totally colour blind patients the second ERG
 - ared in a test condition. Although some investigators tried to use the double
 clinique clinically (Sverak et al 1962) routine employment of it has not been
 ported. In this study double flash technique was applied to patients with
 disturbance from macular and optic nerve disorders. The former showed
 ntly abnormal value of k , whereas the latter showed a normal value. In Figs
 3 the differences of k values are clearly shown between these two groups. It
 that double flash ERG is a useful tool in the differential diagnosis of visual
 rance from macular and optic nerve diseases. As shown in case 2 and 3
 nality of k might correspond to the functional impairment of macula and k
 between the stimulus intervals of 200 mseconds and 500 mseconds seemed im-
 portant. Double flash ERG is very convenient way to measure the cone
 n quantitatively though at present information about it is not enough. Other
 -tages of double flash ERG are that it is easy to perform clinically and not
 onsuming. Because the recorded ERGs are considerably large the measure-
 - of ERG amplitudes are always easy and accurate without employing
 -ring technique. It is concluded that the double flash ERG is a very useful
 ophysiological method to separate cone and rod function and that it can be
 oved as a routine examination for patients with suspected cone dysfunction.

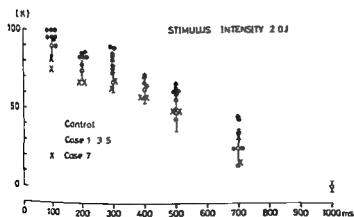


Fig 8

k values of the case 1, 3, 5 and 7 plotted with control values. Stimulus intensity was 2.0 joule

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VISUAL EVOKED RESPONSES TO PATTERN REVERSAL STIMULATION IN CHILDHOOD AMBLYOPIA

BY

PETER WANGER and HANS E. PERSSON

Visual evoked responses to monocular and binocular pattern reversal stimulation were recorded in ten normal and twentythree amblyopic children. In twenty of the twentythree children with amblyopia the responses were found to differ from those in the normal group in one or several of the following parameters: standard deviation of amplitude, standard deviation of latency, amplitude increase to binocular stimulation. The described method can be of value as an aid in diagnosis of amblyopia.

Key words: visual evoked responses, pattern reversal stimulation, monocular and binocular stimulation, amblyopia, childhood.

Childhood amblyopia is considered to be curable if diagnosis is made and treatment started at an early age (Bishop 1975). An objective technique for diagnosis would obviously be of value as the results from psychophysical examination of young children are not always reliable.

The recording of visual evoked responses (VER) offers a possibility of objective examination of the visual system. Judging from several studies (e.g. Spekreijse et al 1972, Arden et al 1974, Sokol & Shatzman 1976, Levi & Harwerth 1978, Srebro & Wanger & Nilsson 1978) amblyopia can be demonstrated by VER examination provided that the stimulus is adequately chosen, i.e. consisting of a reversing checkerboard pattern (Spekreijse et al 1972, Arden et al 1974, Levi & Harwerth 1978) with small checks (Sokol & Shatzman 1976), possibly also low contrast between squares (Spekreijse et al 1972, Barnard 1978) and low reversal frequency (Srebro 1978, Wanger & Nilsson 1978).

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In the present study these aspects of the stimulus parameters have been taken into consideration and the results from examination of 23 children with strabismic amblyopia and 10 normal children are reported.

Material

The normal group consisted of 10 healthy children, age 4-6 years, with normal visual acuity and without history of squint or subnormal vision among their siblings.

The pathological group consisted of 23 children, age 4-6 years, who were referred to ophthalmological examination because of subnormal visual acuity found at the general health examination, which is performed on all children 4 years of age in Sweden at the Children Health Care Centers. All cases in the group were examined by an experienced ophthalmologist who made a general ophthalmological examination including retinoscopy with atropine mydriasis. Refractive errors were corrected and the prescribed glasses were also used during the recordings.

Methods

Stimulation

Pattern reversal stimulation was obtained with a commercially available TV pattern generator (Medelec). A checkerboard pattern was presented on a standard 19-inch TV screen at the patient at a distance of 1.5 m. The whole stimulating field corresponded to a visual angle of 15.2° and each square subtended a visual angle of 1° . The average luminance of the screen was 40 cd/m^2 with a contrast between squares of 30%. Monocular and binocular (dioptric) stimulation was used with a pattern reversal frequency of 1.5 Hz.

Recording

The VER was recorded between an electrode applied to the scalp 5 cm above the ear on the midline (Oz) and a midfrontal reference (Fz). The signals were fed into a preamplifier with low and high frequency filters set at 0.8 and 80 Hz, respectively. Responses to 100 pattern reversals were averaged on a Medelec DAV 6. Analysis time was 300 ms. Latency of the response was defined as the time from the pattern reversal to the peak of the early component. The amplitude was measured from the preceding negative peak to the trough of the early positive wave.

The statistical significances were assessed by Student's *t*-test unless otherwise stated. A *P* value of < 0.05 was considered significant.

Results

Group

Representative VERs from a normal child are shown in Fig. 1. The VERs had a similar waveform with similar latency and amplitude to stimulation of each eye and increased amplitude to binocular stimulation. Table I presents the values of VER parameters in the normal group. Due to the interindividual variation in amplitude the analysis is based on the comparison of amplitude to stimulation of the left eye and to monocular versus binocular stimulus presentation in the individual. Latency and waveform are fairly constant between individuals (LeVitt et al. 1976).

The VERs in the normal group were found to fulfill the following criteria:

- 1. Amplitude difference of amplitude less than 30% (mean + 2 sd) of the largest response to monocular stimulation.
- 2. Amplitude difference of latency less than 5 ms.
- 3. Increase of amplitude to binocular stimulation more than 25% (mean - 2 sd) of the largest monocular response.
- 4. Six of the ten normal subjects had a late positive component (Parker & Salzen 1977) latency > 200 ms was recorded. When present this component was symmetrical to stimulation of either eye but sometimes disappeared with binocular stimulation.

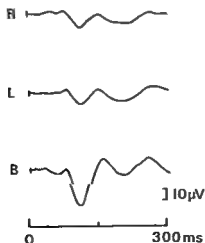


Fig. 1

R from a normal individual (subject No. 3). Note symmetry of amplitude and latency and amplitude increase to binocular stimulation.

Table I
VER parameters in the normal group.

Subject No	Age (years)	VER amplitude (μ V)			VER latency (ms)			Relative difference	
		O D	O S	Binoc.	O D	O S	Binoc.	Side difference (%)	Binocular (%)
1	6	8	8	14	112	116	120	0	-
2	4	10	9	17	110	109	111	15	7
3	4	6	5	11	110	106	109	17	1
4	4	40	36	61	112	113	114	10	-
5	4	15	13	32	109	108	109	13	1
6	4	10	13	21	107	107	105	3	-
7	4	13	13	23	106	106	110	0	-
8	4	13	10	30	112	110	114	22	22
9	5	12	10	17	111	110	109	1	0
10	5	16	16	29	113	114	114	0	-

Pathological group

The patients were ranked according to the side difference of visual acuity expressed in minimum angle of resolution (MAR) which corresponds to the inverted logarithmic visual acuity measured on the Snellen chart (see Table II). Based on this ranking the pathological group was divided into two subgroups P I and P II. The former subgroup P I had side differences of MAR less than 1.7 and the latter subgroup P II had side differences of more than 3.2. The difference between subgroups was significant (see Methods).

Positive cover test indicating squint occurred in 36% (4/11) of the P I group and in 92% (11/12) of the P II subgroup. The difference between the group occurrence of squint was also significant ($P < 0.05$; χ^2 -test with Yates correction).

Representative VERs from an amblyopic patient are shown in Fig. 4. The amplitude of the early VER component to stimulation of the right eye was less than corresponding component of the left eye. The amplitude of this component was not significantly increased with binocular stimulation.

The VERs of the pathological group (see Table II) were analyzed with respect to the above mentioned criteria and the mean values and SD in each group are given in Table III.

There was only one significant difference between the normals and the pathological subgroup P I, i.e. binocular amplitude which was lower in the P I group.

The P II group differed from the normals in all three analyzed criteria.

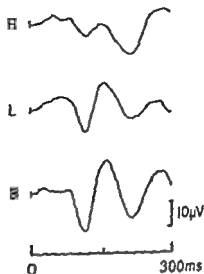


Fig 2

VER from an amblyopic patient (case No. 25). Note marked side asymmetry of the early component and low amplitude increase to binocular stimulation.

side difference of amplitude and latency, and lower amplitude increase to binocular stimulation.

When the PI and PII groups were compared significant differences were observed in side difference of amplitude and latency. No clear correlation was found between the degree of side difference in MAR and the magnitude of difference of VER amplitude when these parameters were compared in 22 cases.

In both pathological subgroups a significant prolongation of latency (7 ms) was found when the responses to stimulation of the better eye were used.

Table III

Mean and SD of analyzed VER parameters of the normals (N) and the pathological PI and PII subgroups.

	N	PI	PII
Number of persons	10	11	11
Side difference of amplitude	$11 \pm 9\%$	$21 \pm 3\%$	11
Side difference of latency	$\pm 1 \text{ ms}$	$3 \pm 1 \text{ ms}$	11
Binocular amplitude increase	$18 \pm 2\%$	$31 \pm 31\%$	

corresponding responses in the normal group. No such difference was found regarding amplitudes.

III of the 23 pathological cases (43%) a late positive component was recorded in stimulation of either the better or the amblyopic eye or both. The late component usually remained with binocular stimulation.

Discussion

Several investigators have studied the VER in amblyopia (for review see Srebro 1978). The changes connected with amblyopia are most easily demonstrated if the stimulus is a reversing checkerboard pattern (Spekreijse et al. 1972; Arden et al. 1974; Levi & Harwerth 1978) containing small squares (Sokol & Shatenian 1976; Wanger & Persson 1979). Reversal frequency should not be too high (Srebro 1978; Wanger & Nilsson 1978). Low contrast between squares may increase the sensitivity of the method (Spekreijse et al. 1972; Barnard 1978).

In our experience, based on recording of transient responses, the use of very small checks (6') or contrast rates below 20% increases the interindividual variability and makes the evaluation of the responses much more complicated (Wanger & Nilsson 1979). For this reason we have chosen a checksize of 12' and a contrast rate of 40%. The 2 Hz reversal frequency allows separation of the early and the late component (Parker & Salzen 1977).

With the described method abnormal VER was obtained in 87% (20/23) of the children with clinically diagnosed amblyopia. The three children with normal VER (Nos. 8, 9, 19) were found to have hyperopic astigmatic refractive errors and needed rapid normalization of visual acuity after glass correction and occlusion treatment. These children presumably had a purely accommodative strabismus without established sensory adaptation.

In the PI subgroup with low degree of amblyopia (VA = 0.3 or better) the only significant VER abnormality was reduced amplitude increase to binocular stimulation. Two of the 11 patients in this group had abnormal side difference of latency and three had abnormal side difference of amplitude. In the PII subgroup with higher degree of amblyopia (VA = 0.2 or less) the VER differed from the normal group in all three criteria, i.e. side difference of amplitude and latency, respectively, and amplitude increase to binocular stimulation. Moreover, all patients with visual acuity of 0.3 or less in the amblyopic eye (Nos. 11-23) except one (No. 19), i.e. 92%, had abnormal VER. Arden et al. (1974) also found VER changes in amblyopia when visual acuity was below 0.3 (6/18).

It has been proposed that amblyopia is caused by suppression of the image from the afflicted eye, established during binocular fixation and maintained during

monocular fixation (Franceschetti & Burian 1971). The present findings of a large amplitude increase to binocular stimulation could be regarded as a suppression during binocular fixation. Low amplitude and or latency increase to monocular stimulation of the amblyopic eye might reflect a weak or a hypothetical suppressive mechanism also during monocular fixation. The present functional disturbance causing this suppression and resulting in the late changes of the VER may be located anywhere in the visual system either in the retina or the site of VER generators in the cortex or at a cortical locus of VER generators. Functional changes in retinal neurons (Ikeda 1979) and cortical (Hubel & Wiesel 1963; Guillery 1973; von Noorden 1973) and physiological (Hubel & Wiesel 1963; Ikeda & Wright 1976) changes in the lateral geniculate nucleus (LGN) have been described in animals with experimentally induced amblyopia. Since evidence has also been published for the existence of a cortico-retinal projection to the LGN influencing the threshold of relay cells (Schmolesky & Singer 1977) a primary cortical disturbance of function may be operative. Where the primary disturbance is located the present results with reduction of VER amplitude to monocular stimulation of the amblyopic eye indicates an involvement of visual system peripheral to the VER generators in the cortex.

Parker & Salzen (1977) suggested that the late component of the transient VER to pattern stimulation might reflect activation of the slower sustained neurons in the visual pathway (Tolhurst 1975). Ikeda & Wright (1974) argued, that in amblyopia might be due to selective lesion of this neuronal population. If both assumptions are correct one would expect absence of the late component in stimulation of the amblyopic eye. However absence of the late component was not noted with stimulation of the better eye and presence with stimulation of the amblyopic eye in the same patient and vice versa. No definite conclusion about significance of the late component can be made in the present study.

It is known that defocusing of a stimulating pattern results in a gradual decrease in the VER amplitude (for references see Desmedt 1977). Haidich et al. (1977) found a clear relationship between the visual acuity and the mean amplitude of VER in patients recovering from optic neuritis. A similar observation was made by Persson & Sachs (1979) on multiple sclerosis patients with visual acuity impairment provoked by physical exercise (Uhthoff's symptom). In the present study no correlation was found between the degree of side difference in VER and the magnitude of side difference of VER amplitudes when these parameters were compared in individual cases. It cannot be excluded that this lack of correlation is due to factors such as the limited number of patients on each visual acuity level, insecurity in visual acuity testing and greater variability of amplitude in the VER-examination of children. However a clear correlation between visual acuity and VER amplitude may not be present in amblyopia since this condition is

of several types of disturbances on different levels in the visual system as above.

Primary results from re-examination of patients in the PII subgroup after treatment indicated that the latency prolongation noted in some of these patients was persistent while side difference of amplitude and amplitude increase to stimulation was normalized in some patients. If these changes in the VER related to clinical development the method may be useful not only as an aid to diagnosis but also for the prediction of treatment results in individual cases.

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THE INFLUENCE OF HYPEROSMOTIC STRESS ON THE BLOOD RETINAL BARRIER EFFECTS ON THE ELECTRORETINOGRAM

BY

PER TÖRNQUIST¹ and AVI RING

Electroretinographic recordings were obtained from cat eyes before and after intracarotid injection of a hypertonic NaCl solution. It was found that after the injection the c wave decreased in amplitude or disappeared but reappeared within a period of four to six hours suggesting that there is a reversible opening of the tight junctions following osmotic stress.

Keywords: osmotic stress - electroretinogram - retinal pigment epithelium - blood retinal barrier

the blood brain barrier the blood retinal barrier consists of a continuous sheet of cells connected by tight junctions which provide a barrier to intercellular flow. Following osmotic stress the tight junctions can be opened thus giving a logical increase in the permeability of the barrier (Rapoport 1970; Laves & Ort 1976; Törnquist 1979). For the cerebral capillaries at least this phenomenon is reversible (Rapoport et al. 1972).

Primary experiments on pig eyes showed that the retina was heavily stained with blue following a severe osmotic stress due to leakage through the retinal pigment epithelium (RPE) (Törnquist & Ring unpublished observation). In these experiments it was further observed that the c wave of the electroretinogram which is known to originate from the RPE disappeared. If there were a

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reversibility of the opening of the RPE corresponding to that found earlier. We could expect the c wave to reappear. The purpose of the present study was to study the behaviour of the c wave following a hypertonic solution of moderate osmolality.

Methods

Two cats (BW about 2.5 kg) were anaesthetized with metubromide (Lundbeck ACO, Sweden). The pupils were dilated by topical administration of 0.5% (Mydracyl® Alcon, Texas) and 10% metaxidine (Neosynephrine® Hoechst) to eliminate the influence of iris potentials. The animals were dark adapted for 30 min. ERG recordings were obtained before an injection of hypertonic NaCl into the exposed ipsilateral carotid artery and then during the following 6 h. A control was also recorded from the contralateral eye as a control. The injection consisted of a 2 osm solution given during 60 sec into the cannulated carotid artery. The blood pressure was measured in one of the femoral arteries.

Stainless steel electrodes were used: one inserted into the corneal area, the other partially isolated was advanced through the conjunctiva to behind the pupil of the eyeball. The potential variations were recorded using a differential amplifier.

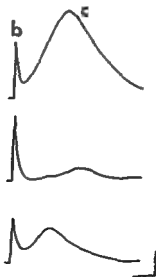


Fig. 1

ERG recordings from first experiment. Before (upper) 15 min after (middle) and 6 h after (lower) osmotic stress. Amplitude calibration 100 μ V. Time scale 1 sec. The b- and c-waves are indicated.

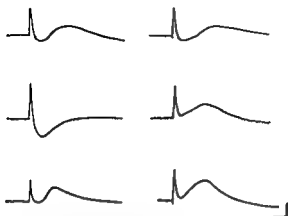


Fig 2

Recordings from second experiment. Left column: experimental eye. Right column: control eye. Before (upper), 30 min after (middle) and 7 h after (lower) osmotic stress. Amplitude calibration 500 μ V. Time calibration 0.5 sec.

A filter with a low frequency cut off at 0.03 Hz was used and the bandwidth was limited to 10 Hz. The signals were amplified 1000-fold and registered on an oscilloscope screen. Traces 0.5 sec in duration were obtained from a 100 W lamp (Astralux Philips) giving an illuminance of 60 lux to the eye.

Results

Small changes in the mean arterial blood pressure were observed during the hypertonic injection. The c wave decreased in amplitude after the hypertonic injection, reaching a minimal level in about 15 min (Fig. 1). A gradual recovery was noted after about 4 h. In the second experiment (Fig. 2) the c wave was completely abolished but began to recover gradually after about 1 h. No significant changes in the ERGs from the control eye (right column) could be detected.

Discussion

The c wave of the ERC originates from the RPE (Noell 1954; Braun & Wiesel 1961; Steinberg et al. 1977). Steinberg et al. (1970) showed that the c wave is mainly generated across the apical cell membrane (facing the photoreceptors). However,

the apical and the basal membranes are electrically coupled, and a pronounced hyperpolarization of the basal membrane of the RPE. The hyperpolarization between the two membranes results in a potential which is the source of the c wave (Oakley & Green 1971).

The magnitude of the c wave is affected by the presence of shunt pathways through the RPE. The importance of shunt resistance for the c wave has been clarified by Oakley (1977). Part of the shunt is the intracellular clefts although these clefts are partially sealed by complexes (Hudspeth & Yee 1973) with a low conductance (Steinberg 1977). Oakley (1977) concluded that without the presence of a change in transepithelial potential upon light stimulation and this would be much larger in amplitude. From this it follows that a tight barrier is a prerequisite for obtaining a c wave in the ERG and that a shunt resistance diminishes the c wave.

The present experiments have shown that the c wave declines or disappears after a 2 osm NaCl injection given into the carotid artery in rats. This is in agreement with experiments on pigs (Tornquist & Ring unpublished). The disappearance of the c wave can be interpreted as a result of a decrease in resistance as discussed above. However a secondary effect of the trauma could be an enlargement of the subretinal space. This effect which also affects the c wave (Marmor 1979) cannot be excluded.

The results are also consistent with the findings of Laties & Rapin (1971) who observed an increase in the permeability of the RPE to fluorescent dyes after osmotic stress. Our observation that the alteration in the c wave is reversible indicates that the defects produced in the RPE heal within a few hours. The effect of a hyperosmolar solution on the morphology of the RPE has been studied by several groups arriving at somewhat different conclusions. According to Laties (1971) the tight junctions are opened while according to Okinaka et al. (1973) the barrier is due to ruptured cell membranes. The reversibility observed in our experiments would suggest that the trauma was relatively mild resulting in partial damage to the barrier between the cells rather than a widespread disintegration.

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FURTHER STUDIES OF THE CHEMICAL SENSITIVITY OF THE OSCILLATORY POTENTIALS OF THE ELECTRORETINOGRAM (ERG)

I GABA- and Glycine Antagonists

BY

LILLEMOR WACHTMEISTER

The oscillatory potentials (OPs) of the mudpuppy ERG were studied to evaluate the effects of GABA and glycine antagonists.

Upon exposing the retina to bicuculline and picrotoxin, blocking agents of putative inhibitory neurotransmitter GABA, all the OPs were completely abolished. The earlier OPs (O₁-O₃) appeared more sensitive to the drugs than the later ones (O₄-O₅). There was no appreciable effect on the rate of sensitivity and saturation level of the b-wave. Low concentrations of strychnine blocking the effect of the putative inhibitory neurotransmitter glycine produced a selective decrease of the amplitude of the OPs. The first (O₁) appeared less sensitive to the drug than the later ones. Higher concentrations abolished all the OPs but also decreased the suprathreshold amplitude of the b-wave.

In conclusion, the present results are in agreement with previous work which states that the OPs appear to have a different origin from the b-wave and were likely generated by inhibitory feed back circuits within retina. The differential sensitivity of the individual oscillatory peaks indicate that perhaps different synaptic activities might underlie the individual oscillatory potentials.

Key words: electroretinogram - oscillatory potentials - chemical antagonists
GABA - and glycine antagonists mudpuppy retina.

There is now good evidence that the oscillatory potentials (OPs) of the electroretinogram (ERG) reflect possible feed back circuits in retina (Wachtmeister & Sjöström 1978). First, the OPs represent radial current flows as the OPs reverse their polarity

microelectrode penetration. Secondly the individual OPs reversed at different times in retina which coupled with different timing suggest a centrifugal sequence of events to give rise to the OPs. Thirdly the OPs showed a differential sensitivity to various putative neurotransmitters suggesting that the OPs are generated by an inhibitory feed back system initiated by the amacrine cells. Inhibitory acting neurotransmitter agents such as gamma amino butyric acid (GABA), glycine and dopamine have all been shown to depress the OPs whereas excitatory putative neurotransmitters such as acetylcholine and carbacholine did not effect the OPs.

There are now five prime neurotransmitter agents which have been firmly established to act in the inner plexiform layer (IPL). These are acetylcholine (Land & Ames 1976; Negishi et al. 1978a), GABA and glycine (see review Neal 1978), dopamine (Dowling & Ehinger 1975, 1978) and one indolaminelike substance (Singer & Floren 1976; Floren 1978).

As the OPs arise in inhibitory feed back synapses in the inner part of retina pharmacological studies might allow further dissection toward determination of their origin. Therefore in the present report extending earlier work some neurotransmitter agents of GABA and glycine were tested. GABA and glycine antagonists have been described to affect the amplitude of the b-wave but no systemic study of the influence of these antagonists upon the OPs has been published (Bonaventure 1974; Pong & Graham 1976, 1978; de Vries & Friedman 1977).

Methods

Preparation

Experiments on *Necturus maculosus* about 25 cm long (Sea Plantations Inc. Mass. USA) were used. The animals were kept in waterfilled aerated tanks maintained at about 6°C in a constantly darkened room until they were used in experiments. The water was changed daily and commercial fungicide (TetraFungi Stop, Tetrawerke, Melle, West Germany) was added to the water to reduce the chance of infection from water mold. Animals selected for experiments were decapitated and pushed under dim red light illumination provided by a microscope lamp covered by Wratten No. 29 filter (Eastman Kodak Inc., <0.01% <600 nm). Following the removal of the eye, the cornea, iris and lens were excised to expose the entire retina. The dissection was carried out under a dissecting microscope. The eye was mounted on a Ringer soaked cotton pad and placed within an anatomically and light shielded cage. A silver/silver chloride electrode was lowered into the vitreous humour which was viewed in dim light under a microscope. Thereafter the retina was allowed to dark adapt for about 5-10 min.

Recording system

ERG was recorded between an indifferent electrode behind the eye and an active electrode in the vitreous. The active electrode was a silver-silver chloride wire insulated to its

up with lacquer. The reference electrode was a coiled silver wire electrode. Potentials were fed into an ac-coupled preamplifier (Neurolog NL 12) with an input resistance of $10^9 \Omega$. Coupling constants of either 1.5 sec or 15 sec were used. High frequency cut-off response was set either at 10 kHz (using a long time constant) or at 100 kHz (using a short time constant). The signals were displayed on one of the channels of a storage oscilloscope (Tektronix type 5115) and photographed by an oscilloscope camera (type C 3 A). The sweep of the storage oscilloscope was synchronized with the shutter of the photostimulator by a triggering circuit. 5 grads from a pulse indicated stimulus duration were amplified and displayed on the oscilloscope.

The amplitudes of the a- and b-waves and the individual OPs were measured from the photographic records. The amplitude of the OPs were measured as the distance between successive troughs of the wavelets and expressed in arbitrary units. The amplitude of the b-wave was measured from the baseline but virtually the same was obtained when the b-wave was measured from the a-wave trough. The amplitudes of individual OPs a- and b-waves were measured from stimulus onset to the peak of individual oscillatory peaks b-wave or negative a-wave.

c Photostimulator

Stimulus light was obtained from a xenon arc lamp (Oxram XB1 Ltd.). The output (Log O) of the system was 1.17×10^{20} photons cm^{-2} sec measured by a Universal Photometer. The stimulus beam was focused on the retina by means of a lens system and covered the whole eye-cup. Intensity of the light beam was varied by density filters. Stimulus duration was regulated by an electromagnetic shutter (Vincent Lab Inc.). Flashes of 75 msec duration delivered at a constant luminance were used.

d Solutions

The drugs were dissolved in an amphibian Ringer's solution (0.5 g NaCl/l, 0.14 g KCl, 0.16 g CaCl₂, 0.3 g hepes/l) and the pH was adjusted to 6.9-7.3. Concentrations between 0.001 mM to 10 mM were tested except for bicuculline and picrotoxin which were either not soluble in 10 mM solutions (picrotoxin) or could not be tested without keeping an acceptable pH (bicuculline). The control Ringer's and the Ringer's with dissolved drug were held in micrometer syringes (Calmont) which were connected to glass tubings. Glass micropipettes with tips of 50-100 μ were connected to the glass tubings. The micropipettes were mounted on micromanipulators (Narishige) to facilitate positioning of their tips close to the vitreous surface. With this method a volume of 1 μ l could be delivered to the retinal surface. The concentrations seen to be effective concentrations applied and not effective concentrations at recording are summarized below and are drawn from observations obtained from a total of 10 experiments.

Results

A. Effects of bicuculline and picrotoxin

Fig 1 A illustrates the ERG in response to full field flashes of 5 msec duration with an intersumulus interval of 30 sec before the administration of bicuculline.

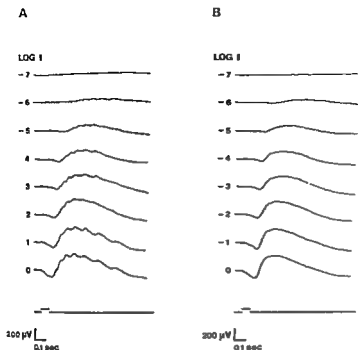


Fig 1

recorded with a long time constant ($\tau = 1.5$ sec) in response to full field stimulus of 75 duration and of different intensities delivered at an interval of 30 sec. A: Before the addition of bicuculline. B: The effect of 0.1 mM bicuculline on the OPs a- and b-wave of RG. The effect appeared within 1 min after the drug was added and gradually faded, reaching a maximum at about 2.5 min when these recordings were taken. A selective suppression of the OPs occurred.

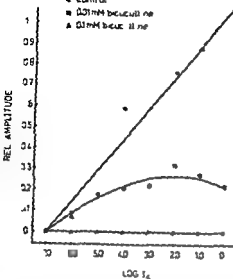
ude OPs could be identified superimposed on the b-wave at intensities as low as -6.0. In response to high intensity flashes the OPs are readily observable. Two minutes after the retina was exposed to 0.1 mM bicuculline all the OPs were selectively suppressed from the b-wave which is clearly seen when the response sets of Fig 1 A and B are compared. In contrast the amplitudes of the a- and b-wave were not affected (Fig 1 B).

Figure 2 A (summed amplitude of the OPs)/Log I (stimulus light intensity) function depicts that the summed amplitude of the OPs increased linearly over a range of 6 log units. There was a linear augmentation of the b-wave over a range of 6 log units beyond which the amplitude levelled off (Fig 2 B). The a-wave increased linearly over the range used in these experiments (Fig 2 B).

Oscillatory potentials

A

- control
- 0.01 mM bicuculline
- ▲ 0.1 mM bicuculline



OPs

- control
- 0.01 mM bicuculline
- ▲ 0.1 mM bicuculline

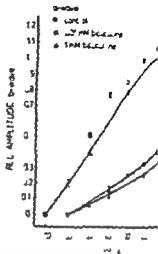
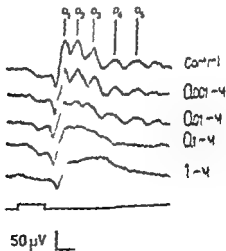


Fig 2

Stimulus-response curves of the OPs a and b-waves of the ERC before and after application of bicuculline. A: Relative summed amplitude of the OPs of the ERC (a) vs $\log I_0$ selectively suppressed the OPs. Higher concentrations (0.1 mM) abolished all the relative amplitudes of the a- and b-waves. The $\log I_0$ curve of the b-wave was unaltered when bicuculline (0.01 mM) was applied. High concentrations also decreased the suprathreshold a-wave.



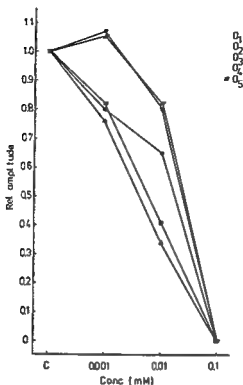


Fig 4

ve amplitudes of the individual oscillatory peaks in relation to different concentrations of bicuculline. The amplitudes are measured from the ERGs shown in Fig 3. The earlier OPs (0s) appeared more sensitive to the drug than the later ones (04s and 05s). All the OPs were abolished when 0.1 mM bicuculline was applied.

Fig 2 illustrates the effect of different concentrations of bicuculline on the stimulus-response functions of the OPs a and b-waves. A low concentration of bicuculline (0.01 mM) dramatically reduced the OPs (Fig 2 A) whereas the V/Log I functions of the a and b waves were virtually unaltered (Fig 2 B). Only a very slight increase of the maximum amplitude of the b-wave was observed after the application of bicuculline. There was no appreciable change of threshold sensitivity of the a and b-waves.

1 mM of bicuculline extinguished all the OPs (Fig 2 A). When higher concentrations of bicuculline (1 mM) were used, a slight decrease of the amplitude of the a-wave was observed (Fig 2 B).

Fig 3 shows the differential effect of bicuculline on the individual oscillatory peaks. Low concentrations of bicuculline (0.001 mM and 0.01 mM) selectively and

have been disrupted either by saturation of GABA receptor with bicuculline or by blocking the action of GABA by the α -cyano- β -methyl-L-glutamate or picrotoxin

Laminar profile studies of the mudpuppy retina have shown that the OPs have their origin in feed back circuits in the inner part of retina (Dowling 1978). The present findings may therefore reflect the properties of these neural pathways. These neural pathways are probably independent of the GABAergic pathways.

The oscillations on the decaying slope of the on transient of the photoreceptor response (P₁R) an extracellular potential recorded in the outer plexiform layer have been shown to decrease in amplitude after treatment with GABA antagonists (Burkhardt 1972; Mooney 1978). A negative feed back between the photoreceptor and the inner plexiform layer has been suggested to be GABA mediated (Burkhardt 1972). There is now also morphological evidence for the existence of an interplexiform cell in the cat retina which constitutes another feedback circuitry of retina (Nakamura et al 1979). Recent electrophysiological studies indicate that GABA may play a role in a feed-back synapse between the photoreceptors and the cones in the cyprinid retina (Wu & Dowling 1978). The feed back circuits responsible for the OPs seem to share this GABAergic pathway with other proposed feed back pathways in the retina.

Another finding of interest is that the OPs were selectively reduced by low concentrations of the convulsant strychnine. Strychnine antagonizes the action of the longer amino acid glycine (Eccles 1964; Curtis et al 1972; Mooney 1978). Thus the effect upon the OPs may be due to the antagonism of the glycine network within the retina.

In a previous study on the mudpuppy retina it was shown that the OPs were selectively reduced when glycine was added to the open eye cup preparation (Dowling 1978). By analogy with the GABA/bicuculline results the OP pathways may have been inactivated either by saturating the glycine receptors with application of glycine or by blocking the effect by the administration of strychnine.

Histochemical as well as physiological studies have suggested that glycine is probably an inhibitory synaptic transmitter and strychnine is a potent blocking agent of glycine in the III of retina (see review by Dowling 1978). The present results support a previous proposal that only inhibitory feed back pathways are involved in the generation of the OPs (Wachtmeister & Dowling 1978).

Recently it has been suggested that glycine also may be involved in feed back pathways which mediate the red light response of the C-type horizontal cells in the carp retina (Wu & Dowling 1979; Belgum & McReynolds 1979). It is therefore possible that the OPs might reflect synaptic activity in the outer plexiform layer. However, such an interpretation is not compatible with the behaviour of the OPs.

studies (Wachtmeister & Dowling 1978) and is unlikely. On the other hand morphological studies have indicated the existence of glycinergic interneurons in the goldfish retina (Marc et al. 1979). The activity of such a pathway would then be more suggestive to underlie some of the potentials.

One observation to be emphasized is the selective and differential sensitivity of the ERG components. First, the OPs were selectively decreased or even abolished whereas the b-wave is hardly affected. Thus, the present findings reinforce the idea that the origin of the b-wave is different from the OPs which suggests different origin of the b-wave compared to the OPs. Secondly, the later OPs (O₄-O₅) were less sensitive to glycine antagonists than the earlier ones (O₁-O₃). The first OP (O₁) was less sensitive to the glycine antagonist than the later ones (O₂-O₄). It has been shown that depolarizing bipolar cells and the on response of the on-off amacrine cells are sensitive to glycine, whereas the hyperpolarizing bipolar cells were more glycine sensitive (Miller & Dacheux 1976; Miller et al. 1977). Glycine has also been shown to selectively depress the off-discharge of the on-off cells and certain features of the carp retina leaving the on-discharge unaffected (Negishi et al. 1977). The existence of separate GABA and glycine releasing amacrine cells has been suggested (Miller et al. 1977). Picrotoxin has been demonstrated to block selectively whereas strychnine was shown to block some features of other ganglion cells (Wyatt & Daw 1976). Picrotoxin has also been shown to alter the receptive field size of both sustained and transient on-off ganglion cells. Glycine altered the temporal organization abolishing the off discharge of the on response in the frog retina (Bonaventure & Wioand 1974). Thus, the differential sensitivity of the OPs may suggest that perhaps chemically different pathways underlie the individual oscillatory peaks.

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THE ALTERATION AND ASYMMETRY OF CUP AND DISC DIAMETERS

BY

BO BENGTTSSON

Increases in cupping with time and differences in cup size between the eyes of one person were studied in a material derived from a general ophthalmic population survey. Cup and disc diameters were measured from fundus photographs. The effect of refraction on the magnification of the eye camera system was compensated for by the use of a simple correction factor.

The disc diameter increased slowly but steadily with advancing years but the rim area remained unaffected and as a consequence the rim breadth diminished. The increase in disc diameter and the decrease in rim breadth seemed to cause a marked increase in cup diameter with ageing.

A multiple regression analysis showed cup diameters to be independent of refraction as well as of systemic blood pressure and only weakly associated with intraocular pressure.

Side differences in cup diameter were strongly dependent on side differences in disc diameter.

Some implications of these findings were briefly discussed.

Key words: cup diameter - disc diameter - age - intraocular pressure - systemic blood pressure - refraction

Theodore Schwartz *et al* (1975) last emphasized that the physiological cup of the normal eye is not a static structure and that greater understanding of age-related changes in this least studied affiliate of the glaucoma triad is needed. New methods for detection of minor changes in the optic disc by comparison of fundus photographs (Goldmann & Lotmar 1977 1978 1979 Bengtsson & *et al*)

) have renewed our interest in this field. The present reinvestigation of existing cross sectional data (Bengtsson 1976) was undertaken as a preliminary time-consuming prospective studies.

Material

Material was derived from a general ophthalmic population survey carried out at Dalby Community Care Centre in southern Sweden from March 1969 to 1970. Based on a directory, invitations were mailed to all persons aged eight or more who had been resident since December 1968 in the suburban village surrounding the centre. Out of 1917 persons invited, 1702 (88.8%) took part in the

survey. Fundus photography was failed in 1620 cases but reliable measurements of cup and disc sizes were obtained from no more than 2334 phakic eyes in 1322 subjects. Six eyes in four patients with suspected or manifest glaucoma were excluded from the present study. A complete set of data was obtained for 2274 eyes in 1287 subjects.

Methods

Visual acuity, tonometry, sphygmomanometric measurement of the systemic blood pressure, subjective refraction in cycloplegia and fundus photography were attempted in every subject. Conventional equipment was used according to a fixed protocol.

Regression analysis was carried out with two standard computer programmes - BMDP and SPSS.

Fundus photographs were taken by an assistant using the modern Zeiss fundus camera with standard magnification (2.5 \times) and Kodachrome II film for transparencies (24 \times 36 mm). The astigmatism-correcting device was used and the camera-extension was preadjusted to the refraction of the eye under examination (Bengtsson & Krakau 1977).

Slides were projected on to a screen at a fixed magnification (16 \times) and traced on ruled paper by the author. Not only differences in colour between the fundus and the film but also other features such as deflections of the vessels were taken into account when estimating the amount of cupping of the optic nerve head. Measurements were expressed in arbitrary units of approximately equivalent to μ m. The geometrical mean of the vertical and the horizontal diameter was used to describe the size of cups and discs. The effect of refraction on the

magnification of the eye-camera system was compensated for by the use of a magnification correcting factor (Bengtsson & Krakau 1977). In non-excavated discs, the rim vessels were considered equivalent to a cup with a diameter of two units.

Results

I Alteration of the optic nerve head with advancing years

The observed disc diameter (d) and cup diameter (c) were used to calculate the double rim breadth ($d-c$) as well as the compact-disc diameter ($\sqrt{d^2 - c^2}$), the diameter of a circle with the same area as the rim. Changes in the means of

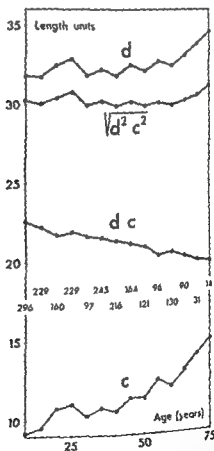


Fig 1

Changes in disc diameter (d), cup diameter (c), compact-disc diameter ($\sqrt{d^2 - c^2}$), double rim breadth ($d-c$) with advancing years. The circles represent the mean numbers, the number of observations in each class. One arbitrary length unit is approximately equivalent to 0.05 mm.

parameters with advancing years in the present material were illustrated in

With the exception of probably insignificant deviations in subjects in their sixties and seventies a more or less linear relationship with age was indicated. The disc diameter increased slowly with advancing years but the mean compact diameter remained unaffected and the mean double rim breadth decreased slightly. The changes in disc diameter and rim breadth combined to cause a small increase in mean cup diameter with age.

The average increase in disc diameter was 0.02 arbitrary units/year. The average decrease in double rim breadth was 0.04 units/year and accordingly the average increase in cup diameter 0.06 units/year. In other words the mean cup diameter increased from about 0.5 mm at 10 to about 0.75 mm at 75 years of age.

Multiple regression analysis of variations in cup size

Disc diameter was independent of the intraocular pressure and the refraction. The fact that cup diameters are unevenly distributed (Bengtsson 1976) and the effect of age had however to be taken into account in a study of factors more closely associated with cup size. We therefore used a stepwise multiple regression analysis in which disc diameter and age were included together with the intraocular pressure (IOP), the systolic blood pressure (SBP) and the refraction (REF) as independent variables.

When disc diameters and age had been added to the regression equation the partial

Table I
Partial coefficients of association with cup diameter

Independent variable	Partial coefficients of	
	Regression	Correlation
Entered		
d	1.16 ($P < 0.001$)	-
age	0.04 ($P < 0.001$)	-
Not entered		
IOP	-	0.08 ($P < 0.01$)
REF	-	0.05 ($P > 0.05$)
SBP	-	0.01 ($P > 0.05$)

added to the regression equation

d = disc diameter IOP = intraocular pressure REF = refraction

SBP = systolic blood pressure

Table II

Observed and corrected variances and coefficients of association between cup and disc
for diameters and their differences

	Observed values for		Corrected values	
	Diameters	Differences	Diameters	Differences
Cup variance (V_c)	26.0	7.0	23.5	6.4
Disc variance (V_d)	11.6	4.9	10.8	4.6
$\sqrt{V_c \times V_d}$	17.4	5.4	16.6	4.9
Regression of cup on disc	1.18	0.77	1.01	0.75
Cup-disc correlation	0.79	0.59	0.85	0.66

Corrected variance = observed variance \times correlation between repeated measurements

Corrected coefficient = observed coefficient \times observed variance / corrected variance

(The pertinent variance is V_d for the regression of cup on disc and $\sqrt{V_c \times V_d}$ for the cup-disc correlation)

correlations of systolic blood pressure and refraction with cup diameter were insignificant and that of the intraocular pressure very low even if statistically significant (Table I)

By the examination of residuals product terms subsamples and polynomials approximations it was possible to exclude major non-linear and non-additive relationships

The regression equation after the second step

$$c = 1.16d + 0.04 \times \text{age (in years)} + e - 28.1 \text{ units}$$

(in which e stands for a normally distributed error term - the cup-disc residual) was therefore considered to give a reasonably accurate description of variations in cup size observed in the present material

III Side differences in cup and disc size

Two diameters are needed to determine one side difference but side differences are less dispersed than diameters. The proportion of the observed variance due to errors of measurement is therefore much larger for side differences in disc than for the diameters themselves. We therefore used the correlation between repeated measurements (0.93 for d and 0.98 for c) to estimate corrected variances as well as coefficients corrected for attenuation in addition to observed ones (Table II)

Even after such correction the standard deviation of the difference between right and left diameter was about 50% of that for the diameters themselves

ent was equally valid for cups and discs (Table II). After correction for variation by errors of measurement the coefficients describing the covariation in cup and disc sizes were almost exactly the same for side differences in diameter as for the diameters themselves. In this respect therefore inter- and intra-individual variations seem to follow the same rules.

Discussion

■ Investigators are said to have reported mixed findings with respect to an effect on the cup-disc ratio and it is generally taught that cupping does not increase with age. (For references see Schwartz et al. 1975.) A closer scrutiny shows that all earlier reports in the literature agree with the present one in showing an apparent increase in cup size with age even if this trend has not always been confirmed by statistical tests for significance.

The use of the cup-disc ratio to describe the size of the cup is based on the assumption that the size of the disc is constant. An increase in cup-disc ratio has therefore seemed to imply a loss of neural tissue difficult to accept in normal eyes. The results of a cross-sectional study like the present one can reflect no more than averages of changes in the population and the range and time-course of individual changes in cup and disc sizes remain largely unknown. It is however difficult to avoid the following interpretation of Fig. 1.

There is normally no loss or gain of neural tissue and the area of the rim remains constant by age. There is however a slow but steady increase in size of (a majority of) all discs. A small increase in disc diameter gives a relatively large increase in disc area necessitating the same increase in cup area in order to leave the rim unaffected. Since the area of the cup is much smaller than the area of the disc the resulting change in cup diameter is larger than the change in disc diameter. Consequently the rim breadth decreases when the disc diameter increases. The amount of tissue thus remains constant in a certain mature nerve head but it varies between different discs and the combined effects of all influences (including age) leave the rim breadth (rather than the rim area) largely independent of disc size (Bengtsson 1976). The multiple regression equation

$$c = 1.16d + 0.04 \times \text{age} + c - 28.1 \text{ units}$$

therefore be interpreted to mean that changes in cup diameter in general more closely parallel those in disc diameter but that ageing also results in a decrease in rim breadth that should be taken into account.

The apparent similarity of cup-disc relations valid for side differences in diameter to those valid for diameters themselves seems remarkable in view of the

opinion that cup and disc diameters are to a great extent inherited (Björ, 1980) while their side differences cannot be inherited at all.

Hessing & Gregersen (1977) have reported a pressure-conditioned distension of the disc causing reversible cupping in early stages of congenital glaucoma. Our results gave no reason to believe that the increase in disc and cup diameters as reported here could be regarded as an effect of the intraocular pressure. The disc is distended by normal pressures in the adult. The changes in cup size and intraocular pressure were however not cumulative and therefore not likely to be irreversible.

The possibility of an acquired enlargement of the optic cup without an optic atrophy reported here calls attention to a similar possibility of cavernous degeneration with extensive loss of nerve fibers in the total absence of atrophy of the optic nerve head (Schnabel 1905). It appears that neither an enlargement nor a diminishing rim breadth should be considered pathognomonic of glaucomatous disease. Our search for the first signs of glaucomatous damage should perhaps be redirected from the documentation of changes in cup size toward early detection of cavernous degeneration.

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THE INHERITANCE AND DEVELOPMENT OF CUP AND DISC DIAMETERS

BY

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The familial resemblances in disc diameter (d) and cup diameter residual (e) were studied in 845 individuals forming 297 families and 1040 pairs of spouses and first-degree relatives. The cup diameter residual was computed as the difference between the observed cup diameter and the cup diameter expected from the disc diameter and the age according to a multiple regression equation. The results for d and e were similar. Spouses did not resemble each other at all. The resemblance between first-degree relatives, on the other hand, was quite substantial. There were no major influences of sex linkage, dominance or common environment and no obvious maternal effects. It was concluded that the familial resemblance was essentially additively genetic in origin. The heritability was estimated at 2/3. The remaining variance was attributed to errors of development. The results can be applied to other surface dimensions of the optic nerve head.

Key words: familial resemblance - cup diameter - disc diameter - heritability - development

Unprejudiced analysis of the familial resemblance has led to doubts concerning the hereditary nature of the intraocular pressure (Bengtsson 1976a) and the blood pressure (Bengtsson et al 1980). The finding of an apparent similarity between the disc relation for side differences in diameter and that for the diameters themselves (Bengtsson 1980) alerted us to the possibility that the hereditary nature of the diameters should be doubted too. The prevailing opinion that the size of the optic cup is inherited dates back to studies on the genetic determination of the cup-disc ratio (For references see Watzman et al 1975). The use of the cup-disc ratio was based on the assumption

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that the size of the disc is constant. The determination of the cup size is therefore seemed equivalent to the determination of all essential dimensions of the optic nerve head. The size of the optic disc is not constant, however, and the cup sizes have to be measured separately in order to determine elementary dimensions of the optic nerve head.

Cup diameters are unevenly distributed and heavily dependent on disc size and age (Bengtsson 1976b, 1980). They had therefore to be transformed for the present purpose. To this end we used a multiple regression analysis to calculate expected cup diameters from disc diameters and age. The difference between observed and expected cup diameters, i.e. the cup diameter residual, is normally distributed and (by definition) independent of disc size and age. The two kinds of influences (errors of measurement included) not common to cup and disc diameters.

We therefore decided to analyse the familial resemblance in disc diameter and cup diameter residual.

The total (phenotypic) variance V_P of a measurable property may be regarded as a sum of separate causal components, e.g. the additive genetic variance (V_A), the dominance (V_D) and environmental variance (V_E).

$$V_P = V_A + V_D + V_E +$$

The heritability (h^2) is defined as the ratio of additive genetic to phenotypic variance

$$h^2 = V_A/V_P$$

Parts of some causal components are common to certain family members. This is true for relatives and spouses to co-vary, i.e. to resemble each other. The covariance depends on the type of relationship.

$$\begin{aligned} \text{covariance}_{\text{husband \& wife}} &= \text{common } V_E \\ \text{covariance}_{\text{parent-child}} &= \text{common } V_E + 0.5 V_A \\ \text{covariance}_{\text{siblings}} &= \text{common } V_E + 0.5 V_A + 0.25 V_D \end{aligned}$$

The degree of resemblance is usually expressed in terms of the covariance as a proportion of the phenotypic variance, i.e. as the regression (or correlation) coefficient.

$$b (= r) = \text{covariance}/V_P$$

where V_P refers to the variance of the independent pair member (for b) or to the geometric mean of the variances of both members (for r).

If the covariance caused by common environment and dominance is so small as to be negligible, the regression of children on one parent (b_{OP}) and the correlation between parents (r_{FS}) become equal to half the heritability.

$$b_{OP} = r_{FS} = 0.5 V_A/V_P = 0.5 h^2$$

In this case the degree of resemblance provides a quantitative estimate of the heritability.

Material

Material was derived from a general ophthalmic population survey carried out at Dalby Community Care Centre in southern Sweden from March 1969 to 1970 (Bengtsson 1976b 1980).

Official identification numbers of the eldest sibling and the eldest child of person were requested in a questionnaire and orally verified at the examination. In accordance with these data 297 families consisting of 845 individuals with the measurements of cup and disc sizes were assembled and pairs of first degree relatives and spouses constructed as detailed later. Only one eye (preferably the left) from each subject was used to estimate the degree of familial resemblance.

Methods

Cup and disc diameters were measured on fundus photographs (Bengtsson 1976b 1977). The cup diameter residuals (c) were computed from the observed cup diameter (C), the disc diameter (d) and the age (in years) of the subject according to the following equation:

$$c = 1.16d + 0.04 \times \text{age} + c - 28.1$$

Analysed by a multiple regression analysis of the initial material (Bengtsson 1980). To describe the degree of familial resemblance we calculated both correlation and regression coefficients. All pairs consisted of two individuals. The part of the dependent member of a pair was always assigned to the older relative and to the spouse. Spouses were included only if they had at least one examined child either. Otherwise we used all pairs that could be constructed in order to obtain the greatest possible precision within the limitations imposed by the already existing material. One person might therefore be a member of two families and could appear both as child and as sibling in one of these. The number of fully dependent observations in our groups was accordingly less than might be inferred from the number of pairs.

The probability that observed values or their differences had arisen by chance was estimated after z transformation of the correlation coefficients. Only fully dependent pairs were counted to ascertain that the coefficients were significant. The total number of pairs was used to ascertain that the differences were not significant.

Results

The results for d and e were similar and will therefore be described together.

The coefficients describing the resemblance between spouses were negative or negative. The coefficients describing the resemblance between pairs of members of the seven fundamental groups of first degree relatives on the other hand were positive and with few exceptions (the coefficients describing the resemblance in cup diameter between brothers and that in cup diameter residual between mothers and their sons) statistically significant (Table 1).

The differences between pairs of father-son and pairs of mother-daughter, between pairs of father-daughter and pairs of mother-son as well as between pairs of brothers and pairs of sisters were not significant. The remaining differences between the coefficients of the seven fundamental groups of first-degree relatives were of the same order of magnitude as those just mentioned. Their statistical significance could not easily be tested however since the involved groups were composed of the same individuals to such an extent that they had to be considered dependent on each other. The observed directions of the differences were opposite to those expected from sex linkage. We therefore felt free to perform

Table 1
Familial resemblance in disc diameter (d) and cup diameter residual (e)
(r = correlation coefficient b = regression coefficient)

	Number of pairs	d		e	
		r	b	r	b
<i>Fundamental groups</i>					
Husband - wife	149	0.02	0.09	-0.19	-0.13
Father - son	158	0.39	0.41	0.23	0.28
Father - daughter	133	0.24	0.26	0.23	0.26
Mother - son	191	0.27	0.30	0.19	0.26
Mother - daughter	165	0.41	0.36	0.30	0.26
Brother - brother	77	0.11	0.19	0.33	0.28
Sister - sister	48	0.49	0.36	0.34	0.28
Brother - sister	119	0.29	0.23	0.19	0.22
<i>Composite groups</i>					
Father - child	291	0.33	0.34	0.24	0.28
Mother - child	356	0.34	0.34	0.21	0.24
Sibling - sibling	244	0.21	0.22	0.17	0.13

Table II

The heritability (h^2) of the disc diameter (d) and the cup diameter residual (e) estimated from observed and corrected values pertaining to the regression of children on their parents (b_{OP}) and the full-sib correlation (r_{FS})

	Observed values for		Corrected values for	
	d	e	d	e
V_P of parents	11.2	8.2	10.4	6.6
$^2b_{OP}$ (n = 647)	0.68	0.50	0.73	0.62
V_P of siblings	13.1	9.3	12.3	7.7
$2r_{FS}$ (n = 244)	0.42	0.54	0.45	0.65
Pooled estimate of h^2	0.61	0.51	0.65	0.63

Corrected $V_P = \text{observed } V_P - V_M$

Corrected coefficient = observed coefficient $\times \frac{\text{observed } V_P}{\text{corrected } V_P}$

Pooled estimate of $h^2 = (2b_{OP} \times 647 + 2r_{FS} \times 244) / 891$

ing of the six fundamental groups of first-degree relatives into three major groups (father-child, mother-child and siblings) in order to obtain an optimal estimate of the overall resemblance within each one of them (Table I).

For disc diameters the full-sib correlation seemed somewhat smaller than the correlations between parents and their children. For cup diameter residuals the degree of resemblance was about the same in all three major groups of relatives (Table I).

As discussed later these findings suggested that the familial resemblance in d and e was essentially additively genetic in origin. The regression of children on their parents and the full-sib correlation could therefore be used to estimate the heritability, i.e. the ratio of additive genetic variance to phenotypic variance (Table II).

The variances due to errors in measurement of the disc diameter ($V_{Me} = 0.8$) and cup diameter ($V_{Mc} = 0.5$) were determined by repeated measurements on a limited number of slides and used to calculate the variance due to errors of measurement in cup diameter residual ($V_{Me} = V_M + 1.16^2 \times V_{Mc} = 1.6$) and thereafter to compare the observed variances and coefficients of association (Table II).

In order to get a single estimate of the heritability based on the available information we pooled the two different estimates of the same heritability that took account of the number of pairs of first-degree relatives used in each one of them (Table II)

Discussion

The principal feature of the results reported here was of course the difference between spouses and first-degree relatives. Spouses did not resemble each other at all. The resemblance between first-degree relatives, on the other hand, was quite substantial.

The disparities between different groups of first-degree relatives were not significant and gave no reason to suspect much sex linkage. There were no signs of marked dominance, obvious maternal effect or strong influences from the environment. We therefore concluded that the observed familial resemblance in disc diameter and cup diameter residual was essentially additive genetic.

The coefficients of association are attenuated by errors in measurement which inflate the variances but leave the covariances unaffected. The variance is inflated not only by the original errors in c but also by the errors in d which are reinforced ($\times 1.16$) and transmitted to a via the expected cup diameter. The coefficients for e are therefore more attenuated than those for d . Given the correlation between repeated measurements on the same slide (0.95 for d and 0.93 for c) we would expect nearly 2/3 of the "true" variance in both disc diameter and cup diameter residual to be inherited (Table II).

The finding that the disc diameter and the cup diameter residual are related about the same degree implies, of course, that other surface dimensions derived from them — like the expected cup diameter, the cup diameter breadth and the cup-disc ratio — are also inherited to that degree. The study therefore confirms earlier studies on the determination of the cup (Schwartz et al 1975).

The remaining 1/3 of the variance is by definition environmental and is not necessarily in the common meaning of the word. Properties of anatomical structures are not easily affected by circumstances during life. Individual and the non genetic variation in dimensions of the eye should probably be attributed to "errors" of development and/or to the apparent absence of common environmental influences reported here in the presence of marked side differences reported elsewhere (Bengtsson 1971) in that direction. Small rim breadths in eyes with signs of previous

... engtsson 1976) and high cup-disc ratio in ex pretermates (Fledelius 1978)
 ... cited as examples of such "errors" of development.
 ... findings in one and the same population of results indicating external
 ... nmental influences on the intraocular pressure (Bengtsson 1976) maternal
 ... on the blood pressure (Bengtsson et al 1979) and errors in development of
 ... tic nerve head (inferred here) demonstrate the potential usefulness of the
 ... ds applied here and suggest that a comprehensive analysis of the familial
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EFFECTS OF SYSTEMIC AND TOPICAL ADMINISTRATION OF METOPROLOL ON INTRAOCULAR PRESSURE IN HEALTHY SUBJECTS

BY

ALBERT ALM and CARL PETER WICKSTRÖM

The effect of a single dose metoprolol tablets or eye drops on intra-ocular pressure (IOP), blood pressure and heart rate was determined in healthy volunteers. The ocular tolerance of 6 weeks treatment with 1% eye drops was also studied. A 100 mg tablet reduced IOP for at least 10 h with a maximum effect after 4 h corresponding to 32% of the pre-treatment pressure. The effects of 25 and 50 mg tablets were less pronounced and of a shorter duration. No consistent effect on blood pressure was obtained, but doses that reduced IOP also reduced heart rate. A short lasting reduction in IOP was observed with 0.5% eye drops while 1.3 and 5% all caused significant reductions. At least 10-12 h IOP was reduced by 13, 20 and 29% 4 h after administration of 1.3 and 5% eye drops respectively. No effect was observed in the contralateral untreated eye and no cardiovascular effects were obtained after administration of the eye drops. 1% eye drops administered twice daily for 6 weeks caused no clinically significant side effect.

Key words: intraocular pressure - beta adrenergic blockers - metoprolol - eye drops - ocular tolerance

During the last 10 years an increasing number of reports have confirmed the observation by Phillips et al (1967) that beta adrenergic blockers reduce intraocular pressure (IOP). Thus significant IOP reductions have been observed for pindolol (Bonomi & Steindler 1975), for propranolol, practolol and acebutolol (Wettrell 1977) and for timolol (Zimmerman & Kaufman 1977). Metoprolol, a selective beta₁ antagonist without intrinsic activity and with only weak membrane stabilising properties (Åblad et al 1973) has also been shown to reduce IOP.

matous eyes both when administered systemically (Alm et al 1978) and by (Ros et al 1978 Kriegstein 1978). The IOP reductions obtained in these were large enough to be of clinical interest. Thus the present study was taken to obtain further information on the long term tolerance to metoprolol drops and on dose response relationships for the IOP reducing effect of metoprolol tablets and eye-drops in healthy subjects.

Methods

Participants were healthy without any known previous ocular disease. The study was divided into three parts.

Dose-response metoprolol tablets

Healthy subjects: 8 men and 2 women aged 18–36 years participated in the study. The study was designed as a randomized double masked single-dose study. The investigators determined IOP by applanation tonometry and the other parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) by standard clinical methods after at least 5 min rest.

On 4 different days with a wash-out time of at least 1 week metoprolol was administered orally as tablets containing 0, 25, 50 or 100 mg metoprolol at 8 a.m. IOP was determined at $T = -\frac{1}{2}$, 0, $\frac{1}{2}$, 1 and then hourly until $T = 10$ h. IOP was determined in one eye only: randomized right or left. SBP, DBP and HR were determined at $T = 0, 1, 2, 4, 6, 8$ and 10 h. Venous blood for analysis of metoprolol was sampled at $T = 0$ and 4 h.

Dose-response metoprolol eye drops

Subjects participating in this study were the same as those that participated in study A. The study was designed as a randomized double masked single-dose study. IOP, SBP, DBP and HR were determined as in study A.

On 4 different days with a wash-out time of at least 72 h metoprolol was administered as eye drops containing 0, 1, 3, 10 or 30% metoprolol at 8 a.m. One eye only was treated while the other eye received one drop of the vehicle. IOP was determined for both eyes at $T = -\frac{1}{2}, 0, \frac{1}{2}, 1, 2$ and then every 2 h until $T = 12$ h. SBP, DBP and HR were determined at $T = 0$ and 2 h and venous blood for analysis of metoprolol was sampled at $T = 0$ and 2 h.

Long-term tolerance to metoprolol eye drops

Healthy subjects: all female aged 19 to 42 years participated in the study. Each participant was furnished with two bottles of eye drops marked right eye and left

eye respectively. One of the bottles contained 1% metoprolol while the other bottle only contained vehicle. Neither the participants nor the examiner was informed of which bottle that contained the drug. The participants applied the eye drops twice daily for 42 days. The bottles were refilled with fresh solution after 4 weeks.

Ocular examination. Visual acuity and refraction, tear production (Shirmer's test), corneal sensibility (aesthesiometry) and pupil diameter (in a Goldmann field) were determined before and after 6 weeks' treatment with metoprolol. A general ocular examination with a slit lamp microscope was performed before and after 4 weeks' treatment and at least twice during the treatment period.

Recording of subjective side effects. Each participant was requested to make recordings of possible ocular discomfort. Thus the absence or presence of a stinging, itching, ocular irritation or visual disturbance should be recorded for each eye. The following grading system was used:

0 = none 1 = slightly but not irritating 2 = marked irritation but unendurable 3 = unendurable

Results

For those participating in the dose response studies the following pre-treatment values were obtained: IOP 14.3 ± 0.6 mmHg, SBP 113 ± 3 mmHg, DBP 72 ± 2 mmHg and HR 64 ± 2 (Mean \pm SE, $n = 10$).

A Dose response metoprolol tablets

IOP. The reduction in IOP observed at various times after administration of the drug was obtained by comparison with IOP at $T = 0$. IOP at $T = -1$ did not differ from IOP at $T = 0$. The results are presented in Table I. No effect on IOP was obtained with placebo while 20, 50 and 100 mg tablets all reduced IOP. A significant effect was obtained 2.5 h after administration of the drug. The duration and magnitude of the effect increased with increasing doses and a significant effect that lasted during all 10 h was observed only for 100 mg. At all times of observation the mean reduction obtained with 50 mg was less than the one obtained with 100 mg. This indicates that a larger dose than 50 mg is required to obtain a significant effect on IOP.

HR. The reduction in HR was calculated as for IOP. The results are presented in Table II. Short lasting significant reductions were observed for 20 and 50 mg while 100 mg resulted in a reduction in HR that lasted at least 10 h.

Table I
Reductions in IOP after metoprolol tablets

0 mg			25 mg			50 mg			100 mg		
Mean	SE	P <	Mean	SE	P <	Mean	SE	P <	Mean	SE	P <
-0.8	0.6		0.7	0.6		0.6	0.5		1.7	0.4	0.005
-0.1	0.4		0.1	0.6		1.2	0.4	0.05	2.5	0.8	0.02
0.6	0.7		1.0	0.9		1.6	0.6	0.05	4.9	0.5	0.001
-1.1	0.8		1.3	1.0		2.8	0.6	0.001	3.5	0.7	0.005
-0.9	0.6		2.7	1.0	0.05	2.1	0.8	0.05	4.8	0.7	0.001
0.7	0.7		2.3	1.0	0.05	2.6	0.9	0.02	4.4	1.1	0.005
-0.4	0.6		0.5	0.8		1.8	1.0		4.2	0.5	0.001
0.1	0.7		1.1	0.9		2.3	0.8	0.02	3.6	0.9	0.005
-1.1	0.8		0	0.8		1.9	1.0		3.6	0.9	0.005
1.2	0.8		0.2	0.9		2.1	0.7	0.02	3.5	1.0	0.01
-1.0	0.8		-0.1	0.9		1.3	0.7		2.4	0.4	0.001

and DBP. With the differences in SBP and DBP calculated as for IOP and HR significant increments and reductions were observed at various times for all (including placebo) but no consistent reduction was obtained for either SBP or DBP with any dose.

concentration of metoprolol. All pre treatment samples were negative. Significant levels of metoprolol were observed 4 h after administration of 25, 50 and 100 mg tablets. The obtained serum levels were 71 ± 23 , 150 ± 47 and 310 ± 85 ng/ml respectively. No correlation was observed between individual serum concentrations and effect on HR or IOP.

Table II
Reductions in heart rate after metoprolol tablets

0 mg			25 mg			50 mg			100 mg		
Mean	SE	P <	Mean	SE	P <	Mean	SE	P <	Mean	SE	P <
3.6	1.2	0.05	9.3	1.6	0.001	7.8	1.6	0.005	9.0	2.1	0.005
-1.4	0.8		5.8	3.0		9.6	2.3	0.005	8.2	3.2	0.05
-2.2	2.3		3.2	2.5		3.6	3.7		6.8	2.3	0.02
-0.6	2.0		0	2.6		-1.0	3.7		4.0	2.6	
-1.8	2.4		0.4	2.3		5.2	3.2		7.8	2.7	0.02
3.6	1.7		4.4	2.3		7.2	2.5	0.02	8.8	3.3	0.025

Table III

Difference (IOP in untreated eyes - IOP in treated eyes) after metoprolol

Time h	0.5%			1%			3%			Mean
	Mean	SE	P <	Mean	SE	P <	Mean	SE	P <	
0.5	0.1	0.6		1.9	0.4	0.001	1.5	0.4	0.01	2.3
1	1.3	0.5	0.005	4.2	0.6	0.001	4.7	0.4	0.01	3.6
2	2.0	0.5	0.005	3.0	0.5	0.001	3.9	0.6	0.001	5.0
4	1.1	0.6		3.0	0.7	0.005	3.4	0.5	0.001	4.1
8	1.4	0.4	0.025	3.0	0.4	0.001	3.3	0.5	0.001	4.0
8	0.6	0.5		2.1	0.6	0.01	2.0	0.6	0.01	4.6
10	1.2	0.8		1.5	0.4	0.005	3.0	0.5	0.001	4.2
12	0.8	0.6		1.5	0.7		2.7	0.6	0.005	1.9

B Dose response metoprolol eye drops

IOP The effect was calculated as the difference (IOP in untreated eye - IOP in treated eye). The results are presented in Table III. All doses of metoprolol reduced IOP. A significant reduction lasting 10 h was obtained with 1% metoprolol, while both 3% and 5% caused reductions that lasted at least 12 h. The magnitude of the effect with 5% was numerically larger than that obtained with 1% at all observations indicating that 1% may not be sufficient for a maximal effect. No significant difference in response was observed between 3% and 5% metoprolol. No reduction in IOP compared to the pre-treatment value was observed in the untreated eye with any concentration of metoprolol used.

HR, SBP and DBP Reductions were calculated as in the study on oral tablets. No consistent reductions were observed for any parameter with any concentration of metoprolol.

Serum concentrations of metoprolol None of the concentrations of metoprolol used in the present study caused a significant increase in the measured serum level after administration of the eye drop.

Side effects No side effects were recorded during the two dose-response studies.

C Ocular tolerance study

Subjective side effects The study was initiated as a double-masked study. One of the participants felt a distinct burning lasting less than 1 min in one of the eyes when the eye-drops were applied. It was always the same eye and when the eye was broken it was disclosed that it was the eye that received eye drops containing metoprolol.

prol None of the participants felt that this moderate transient burning
non was unacceptable
art from this initial burning sensation six participants observed no ocular
in either eye. None of the participants noted any visual disturbance
four had recorded ocular discomfort graded one or two in one or both eyes
side effects were mild and transient. None of these participants recorded
itching or irritation for more than 4 days in any eye and for two of them the
mfort was the same in both eyes
the side effects Visual acuity refraction tear production corneal sensibility
pupil diameter did not change in either eye during the study. Slit lamp
inations of the two eyes were normal before and after the study
one participant petechial cutaneous haemorrhages appeared on both legs on
hird day of the study. She had experienced no previous allergic manifestations
cutaneous haemorrhages were restricted to that part of the lower legs that was
red by her stockings. The participant changed stockings and continued the
y. The cutaneous haemorrhages disappeared.

DISCUSSION

reduction in IOP, HR and BP

reduction in IOP In a previous study 50 mg metoprolol tablets administered three times
caused a 30% reduction of IOP in eyes with glaucoma (Alm et al 1979). The
present study indicates that 50 mg at least as a single dose may be a submaximal
Whether a single dose of 100 mg is a maximal dose or not cannot be
cluded from the present study but the use of larger doses was not considered
easystemic administration of beta adrenergic blockers to patients with glaucoma
ld be of clinical interest only if one could obtain a significant IOP reduction at
e levels with a low or moderate effect on the cardiovascular system. In the
sent study 100 mg metoprolol caused a lasting reduction in HR and although
effect of a single dose on the blood pressure at rest is insignificant long term
atment with 100 mg metoprolol tablets can be expected to reduce the blood
ssure (Bengtsson 1976).

eye drops All concentrations of eye drops used in the present study resulted in
nificant reductions in IOP. With an estimated drop size of 50-100 μ l a 0.5%
ution would contain 2.5-5 mg metoprolol. This dose was not sufficient to cause
etectable levels of plasma concentration or measurable cardiovascular effects. Still
IOP reduction obtained with 0.5% eye drops was very similar to that obtained
th 100 mg tablets. Thus topical or systemical applications seems to be equally

efficient for IOP reductions. The mean IOP was reduced by 23% and 21% respectively 4 h after administration of 3% and 5% eye drops and 10% and 12% respectively. This is similar to the reductions of IOP in healthy volunteers treated with other beta adrenergic blockers. Reductions between 20% and 30% are reported for timolol eye drops (Katz et al 1976) as injections of propranolol and oxprenolol (Sharaf et al 1974) and oral administration of propranolol, pindolol and atenolol (Wettrell & Pandolfi 1975). Since these various beta-adrenergic blockers differ regarding degree of selectivity for beta 1 and beta 2 receptors, intrinsic activity and membrane stabilising properties, it seems clear that these properties play a minor role in determining the effect on IOP. It has been discussed that a mechanism other than a beta adrenergic blockade may be responsible for the reduction in IOP (Neufeld 1979).

Ocular tolerance

In the present study 1% metoprolol eye drops given twice daily for 4 weeks did not result in any unacceptable ocular side effect. However, Van Joost et al (1977) have reported a high incidence of conjunctival oedema and hyperaemia and periorbital dermatitis in patients treated with 1-4% metoprolol eye drops. Thus 11 of 33 patients experienced adverse reactions two weeks to five months after initiation of treatment and in 5 of these 11 patients a positive patch test indicated that this may have been caused by a delayed type of hypersensitivity. The reason for the discrepancy between these results and the results of the present study is not clear. The duration of treatment may be important. In the study of Van Joost et al (1977) only four patients experienced adverse reactions within six weeks, the time used in the present study.

In summary, the results of the present study indicate that metoprolol eye drops may be useful in the treatment of glaucoma. 1-3% eye drops administered twice daily seems to be an adequate dose schedule. However, long term studies on the effect on IOP and the incidence of side-effects are needed before the clinical usefulness can be evaluated.

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REFRACTIVE CHANGE IN ALLOXAN DIABETIC RABBIT EYES CONTROL BY FLAVONOIDS I

BY

SHAMBHU D VARMA HAMED K EL AGUIZY and RICHARD D RICHARDS

The rabbit eye is hyperopic by approximately 4D. The induction of diabetes leads to a further enhancement in the degree of hyperopia. This enhancement is attenuated substantially by flavonoids as inhibitors of aldose reductase. The development of refractive changes in diabetic lens involves aldose reductase catalyzed polyol synthesis.

Key words: diabetes — cataracts — refractive change — bioflavonoids — aldose reductase and polyols

The occurrence of cataracts in certain diabetic strains of animals is well known (Yerganian & Meier 1959 Schmidt Nielson et al 1967 Hackett et al 1961 Kuck et al 1968). The formation of cataracts in these strains and in experimentally diabetic animals is preceded by an accumulation of excessive sorbitol in the lens (Heyningen 1959). This lenticular accumulation of sorbitol is a consequence of the action of the tissue aldose reductase on excessive glucose available in diabetes ($\text{Glucose} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{aldose reductase}} \text{sorbitol} + \text{NADP}^+$). A portion of sorbitol gets oxidized to the ketosugar fructose ($\text{Sorbitol} + \text{NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{sorbitol}} \text{fructose} + \text{NADH} + \text{H}^+$). Thus the lenses of diabetic animals also contain elevated levels of fructose (Kinsey et al 1959 Kuck 1961). An accumulation of the excessive glucose and fructose in the lens fibers and epithelium has been proposed to induce osmotic swelling of the tissue and its ultimate opacification (Kinoshita et al 1962 Chylak et al 1969). Cataract in diabetes is in fact a consequence of aldose reductase catalyzed polyol synthesis and its accumulation has been demonstrated

creasingly by recent *in vivo* experiments. Treatment of experimentally diabetic rabbits with certain flavonoids—a class of compounds inhibitory to aldose reductase (Narita et al 1975; 1976) leads to a delay of cataractogenesis (Varma et al 1977; Juliani et al 1979). This delay by flavonoids is accompanied by a decrease in ocular sorbitol and fructose. It is more difficult to implicate lenticular sorbitol synthesis as incumbent upon the activity of aldose reductase in concert with relevant aspects of tissue metabolism (Van Heyningen 1969; 1976; Kuck & Varma & Kinoshita 1974a, b) in the formation of cataracts in human beings. The degree of hyperglycemia and ketoacidosis, duration of the disease, the age at its onset, dietary factors such as proteins and vitamins, environmental factors such as light and genetic traits are some other possible variables affecting the course of cataractogenesis. In addition, there is considerable variation in levels of sorbitol and fructose in human lens (Pirie & Van Heyningen 1964; Heaf & Galton & Varma et al 1979). The levels attained are however proportionate to blood glucose concentration (Varma et al 1979). A precise evaluation of the role of aldose reductase and polyol accumulation in the development of opacity by *in vivo* use of inhibitors of aldose reductase in human diabetics has not been practical. The early effect of diabetes in man consists of refractive changes. This was first described by Horner in 1873 (Ref. Waite & Beetham 1935). The refractive changes are myopic (Duke Elder 1925; Gwinup & Villarreal 1976) as well as hyperopic (Krisman 1973) and are primarily because of lens changes (Fleisher 1923; Vere & Verel 1954). Whether this manifestation of refractive error is linked with aldose reductase activity is not known although it is presumed that these refractive changes are at the initial steps of cataractogenesis. The purpose of this investigation was to ascertain this possibility using rabbits as experimental models. These studies form the basis of further studies on control of ocular refraction in human diabetics by aldose reductase inhibitors and possibly ultimate prevention of cataracts in such individuals through inhibitors of aldose reductase.

Materials and Methods

Twenty rabbits weighing 800 ± 200 g were used as experimental animals. All animals were without any obvious ophthalmologic lesions. Diabetes was induced by intravenous administration of alloxan monohydrate (5% aqueous) in the dose of 100 mg/100 g weight of the animal. Animals were fasted for 48 h prior to receiving alloxan. Administration of the latter was done under light anesthesia imposed by repeated inhalation of diethyl ether vapor. Immediately after alloxan administration, animals received 20 ml of 10% glucose solution intraperitoneally. This was followed by two further glucose injections—one after 4 h and the other after 24 h of

the alloxan administration. Drinking water consisted of a 5% glucose solution for 48 h. The method was essentially similar to that used by Reddy & Kuroki (1967).

Refractive state of the eye was monitored by streak retinoscopy and cyclopegia. The cyclopegic used was 1% atropine sulphate drops instilled 4 times in the morning in the conjunctival cul de sac at intervals of 90 min. Retinoscopy was performed 1 h (enough for full cyclopegia) after the instillation of atropine drops. Baseline refractive power was ascertained by retinoscopy on five successive days. The readings on the latter three days were constant within 0.5 D and hence provided reliable control data. The meridional refractive value was taken for calculation. The trend of the results was unaffected by the meridians with lower values were considered. Animals restrained in a restraint cage when hand held were fully cooperative. The distance between the person doing refraction and rabbit was 75 cm.

Table I
Material and treatment

Group of Animals	Number of Animals	Treatment
1 Normal control (I)	6	no treatment
2 Diabetic control (II)	6	placeton started 3 days before alloxan administration and continued throughout the experimental period
3 Diabetic prophylactic (III)	6	placeton started 3 days before onset of alloxan
4 Diabetic early (IV)	6	placeton started 1 week after onset of alloxan
4 Diabetic early (V)	6	placeton started 2 weeks after onset of alloxan
4 Diabetic early (VI)	6	placeton started 3 weeks after onset of alloxan

The bioflavonoids quercetin and quercitrin (Varma et al 1975) were used as aldose reductase inhibitors. Quercitrin was used in a 5% solution made in 1% citric acid in HCl adjusted to pH between 8.0 to 8.4. The solution was maintained sterile by membrane filtration. Antibiotics (penicillin 100 units/ml streptomycin 100 mg/ml) and antifungal (amphotericin B 0.25 µg/ml). Quercetin was used as 5% suspension in 5% aqueous glycerol. The inhibitors were administered in a dose of 0.34 mg/g body weight divided between subconjunctival intraperitoneal and oral routes. The subconjunctival daily dose was 0.5 ml of quercitrin solution (25 µg). The intraperitoneal dose consisted of 2 ml of the glycerol suspension of quercetin (100 mg/animal). The oral dose was 150 mg/day/animal derived from administration of the 5% aqueous quercetin suspension. Quercitrin, the more readily available of the two flavonoids with regard to its aldose reductase inhibitory activity is orally available and hence its use was limited for subconjunctival route only. The placebos consisted of 1% cysteine HCl (adjusted between pH 8.0 to 8.4) and aqueous glycerol both fortified with antibiotics and antifungal as described above. No placebo was used for aqueous quercetin administered orally (3 ml). The animals were classified and treated according to the protocol given above (Table I) and retinoscoped for 12 weeks. After this period the lenses were cloudy for accurate refraction.

The animals were ascertained to be normoglycemic before administration of alloxan. Diabetes was evidenced by elevation of the blood sugar level to a minimum 400 mg/100 ml after 72 h of alloxan injection, the average level being 400 ± 100 mg/100 ml. The determination of blood sugar was done on samples obtained from the tail vein and using the enzymatic method involving glucose oxidase and glucose oxidase reaction (Heston 1956). All diabetic animals were checked for the level of hyperglycemia at weekly intervals.

Results

The powers of lenses (diopters) required to neutralize the refractive errors in normal and diabetic control rabbits at various experimental stages have been depicted graphically in Fig. 1. In the group of normal animals the power of the lens required to neutralize the refractive error was approximately 4.0 ± 0.5 D, the refraction in the early part of the experiment being slightly on the lower side. During periods up to 48 h it was also observed to lower the refraction by ± 0.5 D. Therefore the animals were retinoscoped routinely in an unfasted state. The contralateral eye of the same animal differed by < 1 D. Anisometropia exceeding 4 D was rare.

Rendering the animals diabetic by alloxan led to significant changes in ocular refraction. From an average of +4 D recorded before the onset of diabetes the

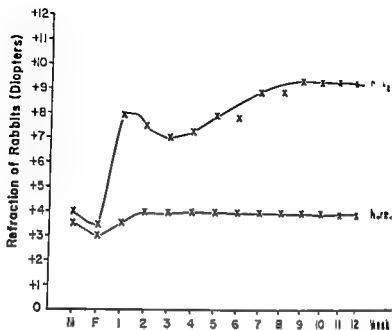


Fig. 1

Effect of diabetes on ocular refraction. Normal. Each point represents an average of 10 measurements. The period of fasting (F) was 24 h. N = nonfasted animals. The number of animals in the diabetic group was similar to that in the group of normal animals. The blood glucose level in the diabetic groups was $400 \pm 100 \text{ mg/dl}$.

refraction rose rapidly to an average of $+8.0 \pm 0.1 \text{ D}$ by the end of the first week after alloxan administration. This deviation was followed by a small drop commencing between the 1st and 2nd week and ending at the 3rd week, the minima being at the 3rd week. Following the fifth week there was a second rise. This rise was however more gradual than the initial one and led subsequently to a plateau $9 \pm 1.0 \text{ D}$. The refraction at the plateau was thus greater than the initial peak observed at the end of the first week ($+8.0 \text{ D}$). The change in the refractive status was thus clearly biphasic though the most pronounced change occurred in the first phase coincident with the initial establishment of diabetes. Elevation coincident with periods in the second phase in comparison with that in the first phase was rather small (1 to 2.5 D) but consistent. Transient vacuoles were also observed during the experimental periods. One of the eyes in the diabetic control group had a vitreal haemorrhage. The changes reported herein were observed consistently including those in three experiments each pilot experiment being done on eleven diabetic rabbits. It was also true in cats and dogs. However it was difficult to render cats diabetic and they were inconvenient to use in these long term experiments.

[illegible]

Norin il control

	Duplicate control	received placebo starting 3 days before allocation injection
III		

Drinking water, flavonoids starting 3 days prior to alcohol treatment

IV. Diabetic given 1 week after allograft treatment

A Diabetic men 6 weeks after allocation in the time in

Diabetics given 11 consecutive placebo pills in the morning

LA CROCE VERDE: UNO DEI PIÙ IMPORTANTI

The doses of β agonists have been given in the text. The bold sugar of all diabetic animals was 25.4(0) \pm mg.

The differences at various stages between the initial refractive state prevalent after treatment in different groups of rabbits administered as described in Table II. Fig. 2 represents the graphic trend of such differences. In normal controls (Group I of Table II) it varied between 0 and 0.5 D. Fig. 2 values in diabetic control (Group II of Table II) is represented by the dotted line. At intervals of 1, 2, 3, 4, 8, 10 and 12 weeks the ΔD s were 4.5, 5.3, 5.2, 5.5, 5.5 and 5.5 D respectively. As would be apparent from the graph, and as shown earlier (Fig. 1) this increase in hyperopia appears to level off after the establishment of diabetes.

The trend of changes in the dioptric power of the eyes in rabbits treated with flavonoids as summarized in Fig. 2 and Table II indicates that this treatment minimizes the effect of diabetes on the refractive power, the effect being more pronounced in groups given flavonoids either before the onset of diabetes or after.

Discussion

The results described in the preceding sections demonstrate that the rabbit is like that of many other terrestrial animals (Duke Elder 1909) is hyperopic compared to the human eye. The extent of hyperopia in the present investigations was approximately 4D. However, the value was somewhat

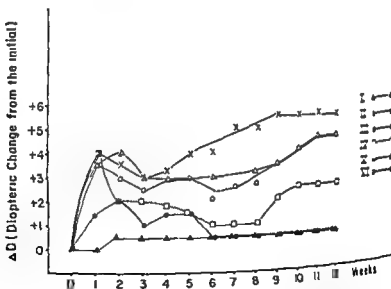


Fig. 2

The numbers indicate the difference in refraction at a particular week from the initial refraction at 0 day. Each group consists of six animals. The figures shown represent the mean of 12 days. The group designations are same as in Table I.

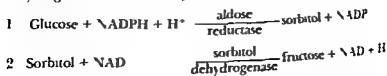
Statistical analysis of the data. Table III (continued) If all given data are taken as correct, the difference between the computed and observed values is less than 1% in all cases.

Groups Compared	1	2	3	4	5	6	7	8	9	10	11	12
1 & 11	11.33	31.8	6.91	7.02	7.92	7.0	7.90	1.71	1.13	9.1	17.77	23.87
1 & 111	9.27	16.85	2.38	7.88	7.11	0	0	0	0	0	0	0
1 & 1V	17.01	7.50	1.00	5	3.33	3.11	3.13	1.22	9.37	1.271	11.76	19.52
1 & V	17.01	17.00	1.31	13.81	11.70	1.15	1.12	9.1	17.14	1.396	21.00	3.52
1 & VI	13.01	13.08	11.76	11.73	8.77	9.7	1.14	1.0	6.12	7.36	10.2	12.92
1 & VII	5.18	17.85	2.12	1.27	5.30	7.0	8.18	1.77	6.14	9.1	11.22	2.02
1 & VIII	0	7.6	1.0	3.18	1	1.0	7.14	5.93	8.8	1.3	16.1	13.02
1 & IX	1.31	2.1	0	0.10	0.1	1.8	1.13	1.31	1.10	2.5	17.6	1.22
1 & X	1	2.71	1.3	0.38	1.8	1	1.13	2.11	1.61	1.12	1.22	2.12

lower side of the mean (by $< 0.5D$) in the first week of the experiment; beginning of the second week the refraction was steady and in relation to the mean. The slight increase in refraction noticed towards the end of the week may be due to the growth of the animals or their adaptation to the conditions. A combination of the two could also be operative. These changes in normal rabbits are however much smaller in comparison to the diabetic animals.

Figures presented in Table II indicate that induction of diabetes led to an enhancement of the extent of hyperopia the ΔD (dioptric difference from initial value at 0 day) being approximately 4 diopters at the end of the first week after alloxan administration (Group II). This change is far greater as compared to that in the normal control group (I). A reference to Table III wherein the data have been quantitatively analyzed would indicate that the t values between groups were highly significant ($t = P < 10^{-3}$) at all points of examination. The results are thus clear in demonstrating that a refractive change in the hyperopic direction is one of the characteristic and predominant manifestations of the diabetic syndrome. This was in conformity with exploratory experiments conducted on three wild pigmented rabbits, six dogs and four cats. Thus, the albino rabbit was considered convenient for the present detailed ophthalmological studies. In the period of examination, diabetes inflicted hyperopia, once established, rather narrowly (Fig. 1) so much so that finally the refraction was observed to follow a plateau covering a range of 1.2 diopters with a median value of 9D. The consistency in the range of variation is in keeping with the range over which the weight of the animals and their blood glucose varied. The blood glucose of the diabetic animals varied within 200 mg per cent (mean 400 ± 100 mg%). The weight of the animals varied by not more than 200 g.

The geometric and the physico-chemical factors which might lead to the observed refractive changes in diabetic animals are not well understood at the present time and further studies are needed in this direction. However, since the treatment of diabetic animals by flavonoids decreases accumulation of sorbitol and fructose in the lens (Varma et al. 1977, 1978, 1979), excessive refractive changes observed in diabetic animals not given flavonoids (Group II) in comparison to diabetic animals given flavonoids (Group III) suggest that development of hyperopia in diabetes may be a consequence of excessive synthesis of sorbitol and fructose. Above polyol and the ketosugar are synthesized by the following two reactions (Heyningen 1959; Kuck 1961):



aldose reductase ■ the first enzyme in the above series and its activity ■
 ed by flavonoids (Varma et al 1975) it appears that this enzyme participates
 ating refractive changes just as it does to initiate cataractogenesis. The
 tive change thus may be an essential event linked with the genesis of lens
 y in experimental diabetes. A comparison (Table III) of refractive changes
 cur in control diabetic animals (Group II) and that in diabetic animals treated
 lavonoids (Group III) indicates that the attenuation of refraction by flavonoids
 . diabetic animals (Group III) is quite substantial and remains highly
 icant throughout the experimental period (t is always 9.0 $P < 10^{-3}$). The
 opic peak attained after 1 week of diabetes in the latter animals (Group III) is
 0% of that in the control diabetic animals (Group II) treated with placebo. In
 quent periods the refraction in Group III almost normalized though they all
 aimed a *consistent hyperglycemia equivalent to that in the control diabetic*
animals. The findings also suggest that the hyperopia is not necessarily linked with a
 ing of blood sugar as often contended (Duke Elder 1925).
 ament of animals (Group IV) when started at the peak of hyperopia
 ring one week after the onset of diabetes also led to a significant return
 ds the baseline refraction (Group I). Following one week of such treatment
 tion in diabetic Group IV fell down to half of that existent before treatment
 was followed by a further decline towards normal till at the end it stayed close
 proximately half of that in the diabetic control (Group III).
 hough commencement of treatment at periods 2nd (Group V) and 3rd week
 p VI) after the onset of diabetes also resulted in a lowering of hyperopia this
 ase was much less striking as compared to that in Groups III & IV where
 tent was started *either 3 days prior to the administration of alloxan (III) or 1*
after alloxan administration (IV). Thus an earlier treatment leads to a better
 nous. In addition these observations are in conformity with the earlier reports
 ing reversibility of galactose cataracts in rats if the galactose from the diet is
 ved well before the advanced stages of cataracts have reached.
 ■ salient difference between these animal studies and the human reports
 sts of the type of refractive error. In human diabetes such an error can be
 rds hyperopia as well as myopia (Bellows 1935) although literature suggests
 myopia is more common. Further studies are therefore in progress to relate
 xholol induced changes such as hydration and the concomitant alterations in
 etric parameters of the lens in reference to its converging power. The effect of
 etes will obviously be algebraic to the physico-chemical and biometric events. A
 uoco-chemical effect may consist of *dilution of the lens constituents resulting in*
l of the refractive index the dilution being due to sorbitol induced hydration.
 fall in refractive index is likely to be compensated by an increase in refraction
 use of geometric alteration consequent to swelling. If the geometric alteration

is low hyperopia will predominate. On the other hand if it is the fall in refractive index because of dilution the result can be due to heterogeneity of the lens segmental nature of its hydration in lens and variation in its elasticity with age are other complications. These are currently being studied in this laboratory. Nevertheless the present results indicate that the initial event in altering the refractive index of the lens and consequent biometric change may involve aldose reductase and is probably by the use of pharmacological agent such as flavonoids and other inhibitors of this enzyme. The susceptibility of human lens polyol accumulation to aldose reductase has been previously demonstrated (Chylak et al 1979 Varma 1979).

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ON THE OPTICAL MEASUREMENT OF CORNEAL THICKNESS

1 Optical Principle and Sources of Error

BY

THOMAS OLSEN CARSTEN BONIELSEN and NIELSEHLERS

The optical principle for the estimation of corneal thickness with a currently employed method is considered. The theoretical relationship between the apparent and true corneal thickness is described in a simple manner. Based on the theoretical results it could be confirmed that physiological variations in refractive index and radius of the cornea induce an insignificant error in the corneal thickness estimate. The theoretical relationship between apparent and true corneal thickness was compared to the performance of two currently available pachometers (Haag Streit and Zeiss). The actual reading of the pachometers was found to be lower than the theoretical reading in a non linear error amounting to about 0.010 and 0.100 mm at a true corneal thickness of 0.50 and 1.00 mm, respectively.

Key words: corneal thickness — pachometer — optical principle

Several methods have in the past been proposed for the optical measurement of corneal thickness (Ehlers & Kruse Hansen 1970). Today, one of the most widely used methods is based on the optical principle originally described by Javal for the measurement of chamber depth. The optical principle worked here is also for the measurement of corneal thickness.

The increasing use of corneal thickness determination in ophthalmological practice places an increasing demand on the accuracy and reproducibility of the method. From a theoretical point of view, the optical properties of the cornea makes it almost a perfect organ for optical measurements. However, the variations in values reported by different investigators still differ considerably. The desire for as much standardization as possible in this field is therefore evident.

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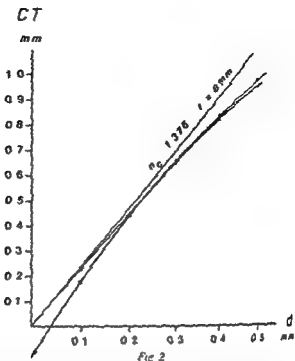


Fig. 2

The theoretical relationship between apparent (d) and true corneal thickness (CT) for a standard cornea of $n_c = 1.376$ and $r = 8$ mm (upper curve). The thick curve is a reading of two commercially available pachometers Haag Strøm (curve with vertical dots) and Zeiss (curve with black dots).

The procedure is to measure the apparent thickness of the cornea as observed at a fixed angle and transform this value into a true corneal thickness. This is done in one step with the commercially available pachometers where a reading allows a direct reading of the thickness from the angular displacement of the beam splitter.

The apparent thickness (d) is a function of the true thickness (CT), the refractive index (n_c) and the anterior radius (r) of the cornea. The exact function (for a fixed observation angle) which defines CT as a function of d

$$CT = f(d) \text{ for given values of } r \text{ and } n_c$$

is however not easily calculated. In the following a simple empirical relation is given.

We assume an extremely narrow slit.

With a fixed observation angle ($= 40^\circ$) the posterior limit of the pupil is seen through a point B at the surface of the cornea. A denotes the anterior limit of the section. We now introduce α the angle of cornea corresponding to 48° for $r = 8$ mm and n_c both the apparent (d) and the true thickness (CT) of the cornea can now be expressed in terms of α .

as a function of α

$$\begin{aligned}d &= |AB| \times \cos(\angle A) \\&= 2 \times r \times \sin(\frac{1}{2}\alpha) \times \cos(\angle A) \\&= 2 \times r \times \sin(\frac{1}{2}\alpha) \times \cos(40^\circ - \frac{1}{2}\alpha)\end{aligned}\quad (2)$$

as a function of α

we have

$$\frac{\sin(i)}{\sin(u)} = n$$

$$i = 40^\circ - \alpha,$$

which u can be found

considering triangle APB we have

$$\angle APB = u + \alpha \text{ and}$$

$$\angle ABP = 90^\circ - u - \alpha$$

the relations now give

$$\frac{|AP|}{\sin(\angle ABP)} = \frac{|AB|}{\sin(\angle APB)}$$

$$|AP| = CT = \frac{2 \times r \times \sin(\frac{1}{2}\alpha) \times \sin(90^\circ - u - \alpha)}{\sin(u + \alpha)} \quad (3)$$

graph of (1) can now be constructed by calculating d and CT for a number of different given values of r and n . This can be done by hand but is easily done with the assistance of a programmable desk calculator (see appendix)

theoretical pachometer reading

Fig. 2 shows the calculated relation between apparent and true thickness for a standard cornea ($r = 8.0$ mm, $n_c = 1.376$). Also included is the performance of the Haag Streit and Zeiss pachometer. The latter curves were made by measuring an optical micrometer (Leitz) perpendicular to the pachometer in question with the scale divided optically in two halves by the beam splitter. One half of the scale was then displaced corresponding to 0.005, 0.10, 0.15 mm etc. on the pachometer and the corresponding scale reading of the pachometer recorded for each setting. The pachometer readings are seen to be lower than the theoretical curve especially for higher values. At 0.5 mm of corneal thickness the pachometer curves were about 0.01 mm below. At 1.0 mm the difference was about 0.02 mm. The readings of the Haag Streit and the Zeiss pachometer were very similar except for small values where the curve from the Zeiss pachometer differed from the theoretical curve and intercepted the ordinate at a negative value.

Fig. 3 shows in a larger scale the effect of varying anterior radius and refractive index of the cornea. The error of varying the radius amounts to about 1% when the radius is changed from 8 down to 6 or up to 12 mm. It can be calculated that the radius should be less than 4 mm to induce an error of 5% or more, an error which could never be reached for values above 8 mm.

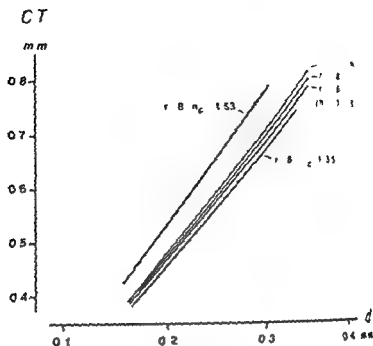


Fig. 7

Theoretical relationship between apparent (d) and true thickness (CT) of cornea's, values of refractive index (n) and radius of anterior surface (r)

The refractive index of the living cornea is not likely to change very much. The most important direction of change is towards lower refractive index if the cornea imbibes water. If the cornea was pure aqueous humour (refractive index 1.336) the error on the corneal thickness determination would be about 4% (upper curve in Fig. 3). This error is of course never reached. If the cornea were to double thickness by intake of aqueous humour the resulting refractive index would be expected to be approximately 1.376 (mean value between the normal corneal refractive index and that of aqueous humour). The resulting error on the thickness determination would be only 2%. Upper curve in Fig. 3 is the theoretical curve for a cornea with refractive index 1.525.

Comments

The theoretical outline of the optical conditions given in the present paper indicates that the corneal thickness measurements are only marginally affected by variations in refractive index and radius of the cornea. Of these factors the

A cornea (anterior radius less than 4 mm) would induce an error of more than

optical performance of the commercially available pachometers were found to be slightly from the theoretical situation by a non linear error which was most at high corneal thickness. The non linear error shown in the present study in Haag Streib pachometer were in good agreement (within 0.01 mm) with correction values given by this firm in a correction table. A similar table is not available for the Zeiss pachometer.

It is puzzling why the pachometer of the latter firm has been constructed so that it gives a negative reading when a negative corneal thickness is obtained.

The non linearity of the pachometer curves is the result of the linear scale of the meters which corresponds to an angular displacement of the two parallel plates of the beamsplitters. From a practical point of view this scale dependent error seems unfortunate. Looking up a correction value in a table each time a reading is taken is a cumbersome procedure. For normal corneal thicknesses and 0.5 mm the error is of course of minor importance but when correct rates of changes in corneal thickness above this value are wanted a correction is necessary. It may be suggested that if the construction of the beamsplitter is not too rigid and a greater reading accuracy is desirable a simple technical improvement may be to change the linear scale into a slightly non linear scale which has been calibrated with the theoretical curve depicted in Fig. 9.

The practical problems concerned with the optical measurement of corneal thickness will be dealt with in a following paper.

Appendix

From equations (2) and (3) this program calculates corresponding values of d and CT when values of r and n by stepwise increasing the value of r until the value of CT reaches 1 mm. In other words the program calculates a number of points on the graph of CT for any given value of r and n .

Programble calculator HP 97

routine B Stores the value of r (in mm)

routine C Stores the value of n

routine D Stores the value (in degrees) by which r is wanted to increase for each pair of d and CT

routine A initializes the program

```

10 STO1 RTN LBL C STO2 RTN LBL D STO6 RTN LBL A DEG RCL1 PRTX/RCL2
11 SPC SPC 0 LBL E STO3 2 CHS 40 + COS/RCL3 = SIN X/2 X/RCL1 X PRTX
12 CHS 40 + SIN RCL2 SIN STO3 CHS RCL3 2 CHS + 90 + SIN RCL3 2
13 X 2 X RCL1 X RCL3/RCL2 + SIN PRTX SPC 1 X > Y GTO7 RTN LBL 7/RCL3
14 GTO E RTN

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INFLUENCE OF TRANEXAMIC ACID AND ACETYLSALICYLIC ACID ON THE THICKNESS OF THE NORMAL CORNEA

BY

THOMAS OLSEN, NIELS EHLERS and THORKILD BRAMSEN

In a masked cross-over study ten normal subjects were treated with tranexamic acid and acetylsalicylic acid one g three times daily for seven days. The central corneal thickness was found to increase in response to acetylsalicylic acid and to decrease in response to tranexamic acid. This finding provides evidence for the involvement of the fibrinolytic system in the regulation of the normal corneal thickness. The endothelial morphology, as seen with the specular microscope, was unchanged during treatment with either drug.

Keywords: acetylsalicylic acid - corneal thickness - endothelium - fibrinolysis - tranexamic acid

Fibrinolytic treatment has been shown to reduce corneal oedema (Bramsen & 1977, Bramsen et al. 1978, Bramsen 1978) while activation of fibrinolysis in eyes with vitreous haemorrhages induces corneal oedema (Bramsen 1978a). Acetylsalicylic acid has been shown to increase the fibrinolytic activity in normal subjects (Menon 1970, Moroz 1977). Presumably because of this effect, acetylsalicylic acid increases the risk of secondary haemorrhages in patients with traumatic intraocular haemorrhage (Crawford et al. 1975). If fibrinolytic factors are involved in the regulation of the thickness of the normal cornea it might therefore be anticipated that acetylsalicylic acid treatment would increase the corneal thickness. The present investigation was undertaken in order to study the corneal thickness response to treatment with the antifibrinolytic drug tranexamic acid and acetylsalicylic acid, which has a fibrinolytic effect in normal subjects. Because activation of

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fibrinolysis in the anterior chamber has been shown to cause damage to the endothelium of bovine corneas (Morton & Turnbull 1964) the appearance of the endothelium was also studied during this investigation.

Subjects and Methods

The effect of tranexamic acid and acetylsalicylic acid on the corneal endothelium was studied as a masked trial. Ten normal volunteers, eight men and two women, age range 24 to 28 years, were given two pill bottles each. One bottle contained Cyklokapron® tablets with 0.5 g tranexamic acid and the other Magnyl® tablets with 0.5 g acetylsalicylic acid. Each subject was instructed to take two tablets three times daily from one of the two bottles for seven days, make a pause of seven days, and then take two tablets three times daily from the other bottle for another seven days. The order of the treatment, tranexamic or acetylsalicylic acid first, was decided and made individually by the subject and not made evident for the examiner. During each treatment period the central corneal thickness was measured daily at 10 a.m. and after noon with a modified Haag Streit pachometer (Ehlert & Sperling 1966). Each measurement was the mean of three determinations each taken as the closest reading on the pachometer. Both eyes were measured and their mean was used as the thickness of the patient. For a trained examiner the standard deviation of single determinations of central corneal thickness with the present technique has been found to be 5–6 µm (about 1%) from a large number of readings on different subjects. After completion of the treatment period corneal thickness curves were drawn for each subject. It was then judged by the examiners which of the two periods were the tranexamic acid treatment period and the acetylsalicylic acid treatment period respectively. After that the true order of treatment was brought to knowledge.

After completion of the masked study three other volunteers were given Cyklokapron® and Magnyl® in the same dosage as above. The corneal endothelium was examined with a non contact specular microscope (Olsen 1979) before and after the seven day treatment periods.

In four other volunteers the central corneal thickness was measured during the periods described above for seven days without any drug treatment.

Results

The masked study

In two subjects it was not possible to judge which of the two treatment periods was the tranexamic acid treated and the acetylsalicylic acid treated, respectively. For

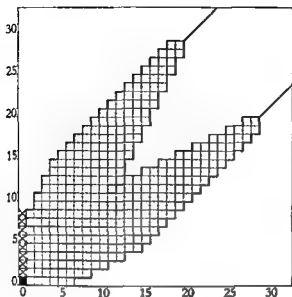


Fig 1

normal analysis chart (95% confidence interval) Vertical step correct judgement on the acetylsalicylic acid and the tranexamic acid treatment periods

During eight subjects the order of treatment judged by the investigators were found to be in concordance with the true order in all cases (Fig 1)

In Fig 2 the mean corneal thickness of the subjects during the tranexamic acid and the acetylsalicylic acid treatment period is shown. In this figure all ten subjects were included. The central corneal thickness is seen to decrease in response to tranexamic acid ingestion. During the first three days a slight tendency towards an increase may be noted after which a decrease is evident. The opposite variations were seen for acetylsalicylic acid.

Corneal endothelium
No specular microscopic detectable alterations in the corneal endothelium was observed either during treatment with tranexamic acid or with acetylsalicylic acid (Fig 3).

Normal corneal thickness variation
In an analysis of variance the mean day-to-day variation (SD) in central corneal thickness of the four subjects was found to be 0.0023 mm. Using this figure the 95% confidence limits for the mean spontaneous variation of ten subjects around

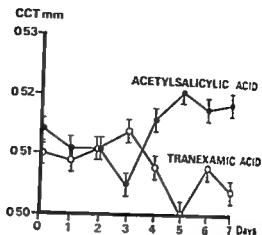


Fig 2

Mean value (\pm SE) of the corneal thickness in ten normal subjects treated with acetylsalicylic acid and tranexamic acid.

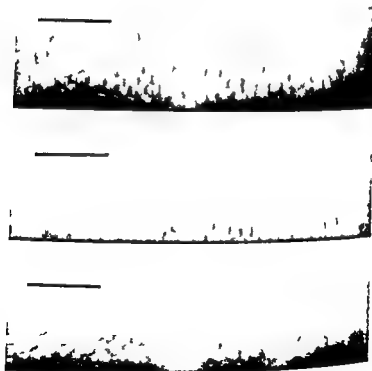


Fig 3

The corneal endothelium in a 25-year-old man before (top) fifth day after two days after (bottom) treatment with acetylsalicylic acid three days after fifth day of treatment a corneal thickness increase of 0.03 mm was found. No specular microscopic appearance is noted. Bar = 10 μ m.

the mean would be expected to be $0.0023 \text{ mm} (= 0.0023 \times t_{0.05}/\sqrt{10}$ three degrees of freedom) (by chance the same value). The observed increase and decrease from the initial mean in Fig. 2 is seen to exceed this limit.

Discussion

The present study has shown that acetylsalicylic acid and tranexamic acid ingested influence the normal corneal thickness.

The decrease in corneal thickness in response to tranexamic acid occurred after 1 to four days of treatment during which a slight increase was noted. The pattern for this biphasic course is not clear. Tranexamic acid has been shown to accumulate in the anterior chamber in a matter of hours after oral ingestion (Jensen 1979). Whether a further accumulation can occur after several days of treatment is unsettled.

However, an important factor which may need consideration in the interpretation of the observed time course is the possible counter reaction of the subject to the treatment. Assuming the involvement of the fibrinolytic system, the observed lag of several days before a decrease in corneal thickness became evident may be explained by an initial compensatory decrease in the fibrinolytic activity of the system. With time this counter regulation presumably becomes insufficient and the effect of the antifibrinolytic treatment then breaks through. If an initial compensation occurs, an initial increase in corneal thickness would be expected.

The above considerations are entirely speculative. The importance of time in the corneal thickness response of the subject was however also found by Bramsen & Jensen (1979) in a study of graft thickness.

The corneal thickness response to acetylsalicylic acid treatment showed a similar biphasic time course. The mirror like effect of acetylsalicylic acid as compared to that of tranexamic acid is strongly suggestive of a mechanism opposite to that of tranexamic acid. This seems consistent with the fibrinolytic activity of acetylsalicylic acid (Venon 1970; Moroz 1977). It has been shown (Nellemann Sørensen 1977) that acetylsalicylic acid enters the anterior chamber. The inability to demonstrate any regular microscopically detectable changes of the endothelium during treatment indicates that if the mechanism was to alter the permeability of this layer, the effect is a physiological one rather than a toxic one.

Whether the effect of acetylsalicylic acid and tranexamic acid on corneal thickness is mediated by an effect on the fibrinolytic system is still unsettled. The fact that acetylsalicylic acid increases and tranexamic acid decreases thickness is however encouraging for a hypothesis of the involvement of the fibrinolytic system in the regulation of the normal corneal thickness.

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CORNEAL THICKNESS AND ENDOTHELIAL DAMAGE AFTER INTRAOCULAR LENS IMPLANTATION

BY

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The corneal thickness and the specular appearance of the corneal endothelium are reported in 100 patients with unilateral intraocular lens implantation. Post-operative time ranged from one to 42 months. An average central endothelial cell loss of uncomplicated cases of 46% (range 1 to 83%) with no correlation with time after the operation was found. A significantly higher cell loss was found in cases with technical complications: shallow anterior chamber or increased intraocular pressure post-operatively. No correlation was found between the corneal thickness and the endothelial cell loss. In two patients, however, with a cell density below 500 cells/mm², a slight increase in corneal thickness was noted. Thirty patients presented a guttate endothelium. In respect of the occurrence of surgical complications the presence of a guttate endothelium was found to be a major determinant of the corneal thickness increase and could be ascribed as a cause of persistent corneal swelling in six of twelve patients with elevated corneal thickness. The progression of guttate changes occurred independently of the cell loss.

Key words: cataract extraction - cell loss - corneal thickness - endothelium - lens implantation - specular microscopy

Cataract extraction combined with an intraocular lens implantation is now becoming an increasingly employed surgical procedure. Along with the good refractive correction it provides to the patient, this insertion of a foreign body into the eye is costly, however. One of the feared complications is persistent corneal oedema, which seems to occur more frequently after lens implantation than after simple cataract extraction (Jardine & Sandford Smith 1974; Pearce 1972, 1975; Duffner

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et al 1976 Baggesen et al 1978) The incidence of corneal oedema in these studies ranges from a few per cent to 13% Other investigators have demonstrated this higher incidence (Binkhorst & Leonard 1977).

In recent years a number of specular microscopic studies have reported an endothelial cell loss associated with lens implantation than without lens implantation after cataract extraction (Bourne & Kaufman 1976 Forstot et al 1977 Sugar 1979 Abbott & Forster 1979 Galin et al 1979) Some studies have not found this higher cell loss (Hirst et al 1977 Binkhorst et al 1977) In these studies the cell loss after lens implantation ranges from 7 to 67% The operative follow up time however also varies considerably Because a reaction of the cell population occurs during the first months after the operation (Hirst et al 1978 Sugar 1979 Galin et al 1979) this makes a direct comparison of studies difficult The lowering of the cell population has been viewed as a concern because a low cell density presumably renders the cornea more susceptible to the development of corneal oedema (Irvine 1956 Capella 1961 Sugar 1979 Bourne & Kaufman 1976a Kaufman 1979)

The immediate post-operative increase in corneal thickness after lens implantation seems to be higher than after simple cataract extraction (Cherg et al 1976 Praeger & Schneider 1977) Because of the quantitative association between endothelial cell loss and immediate corneal thickness increase after cataract extraction it seems that this is to be expected In the above mentioned studies however corneal thickness was found to return to normal levels some months after the operation This is remarkable that even in case of very high immediate increase in corneal thickness after surgery and therefore presumably a high concomitant cell loss, corneal thickness has been reported to return to its pre-operative level (Sugar 1979 Cambiaggi 1976) It therefore seems that the long term effects of endothelial cell loss on the corneal thickness still remains to be shown

The present investigation was undertaken in order to elucidate the effect of endothelial cell loss on the endothelium for the ultimate hydration of the cornea after cataract extraction and lens implantation By this study it was attempted to throw light upon the unsettled question of the information yielded by the endothelial cell loss and its significance for corneal hydration

Subjects and Methods

At the Central Hospital in Nykøbing Falster cataract extraction with lens implantation has been employed since 1976 in patients with senile cataract and more than 60 years of age Indication for lens implantation has generally been found if the patient presented with a history of uveitis no present eye inflammation corneal oedema or corneal dystrophy glaucoma retinal detachment juvenile onset diabetes mellitus and a shallow anterior chamber

surgical procedure was intracapsular cryoextraction with corneal incision. Pre-operative treatment consisted in diamox 500 mg intravenously and eye ball massage. The lens used about was a Federow iris clip lens (manufacturer SM). No special lens coating solution was used to lubricate the lens prior to insertion. To obtain fixation of the lens dry pilocarpine was applied to the wound edge. Normal saline was used as irrigating solution if necessary. Wound closure was done with 8-0 running Dexon® or Vicryl® absorbable suture. All but a few operations were done by one surgeon (E.W.). Post-operatively the patients were treated with pilocarpine eye drops and prednisolone ointments for six and one month, respectively.

In the present study only patients with an unoperated fellow eye without previous trauma or disease other than cataract were included. In this way 140 patients with unilateral lens implantation were selected from the operated series. Eighteen patients had died since the operation. Nine patients could not be traced or did not show up for the present follow up examination. Seven patients were omitted due to poor general condition. Four patients had pseudophakos removed shortly after surgery due to dislocation of the lens and were excluded from the study.

In the present follow up examination corneal thickness measurements were deferred (and also microscopic examination not attempted) in two patients with clear corneas due to fear of the patients to fixate a target. One patient presented with an iridocyclitis on the operated side. Because none of the other patients showed this complication this patient was excluded from the below grouped data (the corneal thickness was 0.500 and 0.510 mm of the unoperated and operated side, respectively and the cell loss was 40% on the operated side).

In all 99 patients 39 men and 60 women in the age range from 69 to 91 years with a unilateral lens implant. Of these two patients had previously undergone intracapsular cataract extraction whereas the rest of the patients had no previous history of disease or trauma in the now pseudophakic eye. The time period from the operation to the present examination ranged from one to 42 months.

Data concerning complications that occurred during or after the operation were obtained respectively from the case record.

Central corneal thickness was measured with a modified Haag Streib pachometer (Ehlers & Ving 1977). Each measurement was taken as the closest 5 µm reading on the scale reading of the pachometer. Single determinations were used the standard deviation of which has been found to be 3-6 µm from a large number of readings on several individuals. Corneal thickness of non-operated eye was taken as control. In what follows residual corneal thickness case refers to central corneal thickness of operated eye minus thickness of non-operated eye.

The corneal endothelium was photographed with a non-contact specular microscope (Ving 1979) in a central area of both sides and 2-3 mm superior in the operated eye. If specular photomicrographs from the central endothelium revealed one or more circular defects in the endothelial reflex larger than two cells width the endothelium was classified as guttate endothelium. By using contralateral eye as control a cell loss was estimated as the residual decrease in cell count from unoperated eye.

Results

Central thickness
In all patients showed a guttate endothelium with bilateral involvement in 28 of 99 cases. Irrespective of the occurrence of surgical complications the presence of a

guttate endothelium was found to be an important factor for the result. As shown in Table I the guttate changes were worse (more numerous and defects in the endothelial reflex) in the operated eye in about two-thirds of the cases which were largely responsible for the increased thickness in this group.

In order to further analyse possible factors influencing the corneal thickness patients were grouped as shown in Table II. If per-operative complications, excessive vitreous loss or difficulty in lens placement had occurred, or if dislocation of the lens had occurred post-operatively the patients were classified as having complications. The mean side difference in corneal thickness for this group was different from the group of uncomplicated cases without guttate endothelium.

Fourteen patients had a shallow anterior chamber post-operatively. The mean difference of this group differed significantly from the reference group (Table II). Six of the patients, however, also had endothelial guttae and when these were withdrawn the mean side-difference diminished to 0.007 (± 0.004) and was not different from the reference group.

The group with increased intraocular tension comprised patients with surgically treated ocular hypertension and patients with a tension higher than 21 mmHg found at the present follow-up examination. Although this group had the highest mean side difference in corneal thickness the scatter was large and the P value just about the five per cent level. Two patients in this group had endothelial guttae.

Table I
■ lateral comparison of endothelial dystrophy and corneal thickness in 30 patients with unilateral lens implants.

Specular microscope appearance	n	ΔCCT (% ± s.e.)
Worse in operated eye	9	+0.03 (+0.01)
Similar involvement on both sides	8	+0.03 (+0.01)
Worse in non-operated eye	3	0.01
Total	20	+0.02 (+0.01)

ΔCCT = central corneal thickness of operated eye minus thickness of non-operated eye (mm).

* $P < 0.01$ by Mann-Whitney U-test compared to second group and

		<i>n</i>	Unilateral eye	Operative eye	Difference	Mann Whitney U test
No surgical complications	- guttate endothelium	10	0.27 (± 0.06)	0.17 (± 0.03)	+ 0.007 (± 0.011)	
	+ guttate endothelium	18	0.10 (± 0.037)	0.564 (± 0.043)	+ 0.021 (± 0.011)	$P < 0.05$
Technical complications		10	0.11 (± 0.015)	0.51 (± 0.034)	+ 0.011 (± 0.012)	ns
Shallow anterior chamber		11	0.290 (± 0.030)	0.512 (± 0.032)	+ 0.07 (± 0.031)	$P < 0.05$
Intraocular pressure > 22 mmHg post-operatively		10	0.3 (± 0.037)	0.81 (± 0.042)	+ 0.038 (± 0.067)	$P \sim 0.0$
Total		99	0.130 (± 0.031)	0.11 (± 0.011)	+ 0.011 (± 0.011)	

indicates significant ($P < 0.05$) difference by Student's paired *t* test

The mean CCT difference was tested against the difference of the first postoperative 1

Two patients fell into two groups. One patient had 11 months of shallow anterior chamber and shallower endothelial keratoplasty and more used myopic laser

ΔCCT

MM

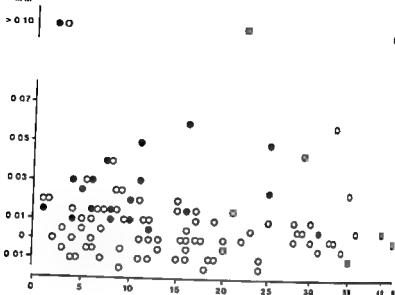


Fig 1

Central corneal thickness of operated eye minus central corneal thickness of unoperated eye (ΔCCT) plotted against time after unilateral lens implantation in 39 patients. \bullet signify patients with guttate endothelium appearing worse on the operated eye. \circ signify normal or non progressed guttate endothelium. Dotted line indicates upper limit of a group without complications and endothelial dystrophy.

dystrophy which, however, was of similar appearance on the two sides, their difference in corneal thickness being only 0.005 mm.

No significant change in corneal thickness with time after the operation was found in the present series (Fig 1). When a group of patients with no complications and without endothelial guttae was selected from the series beyond six months, the mean residual corneal thickness increase was found to be $+0.005$ mm (± 0.015 (SD), $n = 42$) which was significantly different from zero, but not different from similar patients with shorter lens wearing time. Taking a group of patients free of complications and endothelial dystrophy as a reference group, the normal values for residual thickness increase could be said to be ± 0.035 mm (= mean value plus two standard deviations) (dotted line in Fig 1). In this way 11 of the patients could be said to have abnormal persistent elongation of the cornea with a central corneal thickness ranging from 0.045 to 0.200 mm in the operated eye, 0.040 to 0.200 mm different from non-operated eye.

Cell loss before cell loss		Group 1 with cell loss		Group 2 with cell loss		Group 3 with cell loss		Group 4 with cell loss	
	n	Uterine cell loss	Operative cell loss	Cell loss	Cell loss	Cell loss	Cell loss	Cell loss	Cell loss
No surgical complications	19	86 (± 14)	14 (± 19)	- 14.9 (± 19.3)					
	14	2560 (± 399)	19% (± 6.10)	- 48.1 (± 21)					$I > 0.60$
Technical complications	10	2381 (± 479)	10.1 (± 9.7)	- 57.7 (± 17.6)					$I < 0.01$
	19	2,81 (± 419)	9.1 (± 9.2)	- 63.5 (± 14.1)					$I < 0.01$
Intraocular pressure > 12 mmHg post-operatively	8	9.61 (± 40.1)	9.1 (± 11)	- 67.7 (± 1.8)					$I < 0.01$
	91	9.3 (± 38.7)	1.8 (± 1.1)	- 0.9 (± 19.1)					

* Mean cell loss compared with first group lost
 Cell loss compared with the two first groups
 † 3 patients fell into Group 1 and 1 into Group 2. One patient had lens dislocation and increased intraocular pressure.

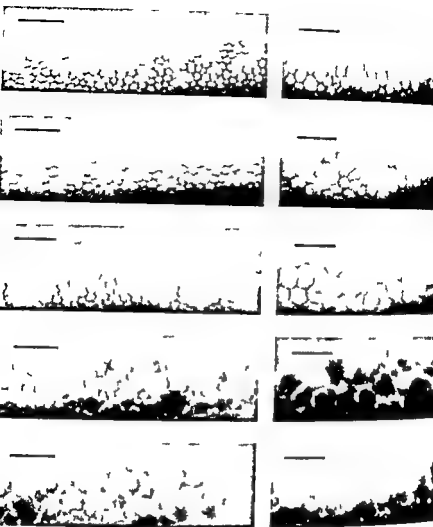


Fig 2

Central corneal endothelium in five patients with unilateral lens implant. Left part of non-operated eye right part operated eye. Two patients at bottom of Fig. 2 show bilateral guttate endothelium with dominant appearance on the operated side. Bar = 10 μ m.

Cell loss

In Table III the data on central endothelial cell densities have been grouped in the same way as in Table II. In eight of the patients bilateral endothelial counts were not available due to poor quality of the photographs (1) excessive changes of the endothelium or marked corneal oedema (7) (see Fig. 1). In the group with no surgical complications identical unoperated cell counts were found

ose patients with or without guttate endothelium Since the central cell loss also not differ these two groups were pooled and compared to the group with plications These groups of technical complications shallow anterior chamber increased intraocular pressure all had a significantly higher cell loss than the group of uncomplicated cases Irrespective of the occurrence of complications mean cell loss of those with or without progression of dystrophic changes was 4% ($n = 15$) and 5.4% ($n = 9$) respectively (non significant)

Fig 3 the central cell loss of uncomplicated cases has been plotted against time the operation No significant relation was found The vertical difference in cell aty i.e the difference between superior and central counts was also not significantly related to the post-operative time ($r = -0.21$ $P > 0.05$) The highest es (about 60% lower density in the superior region) were however found ng the first four months The mean decrease in cell count from the central area he region 2-3 mm superiorly was 24% (± 20) of uncomplicated cases No elation was found to the age of the patient in the present series ($r = 0.19$ $P > 0.05$) A somewhat higher difference in vertical density was found in those ents with a guttate endothelium (33.4% vs 21.5% lower cell count in the nor region for uncomplicated cases with and without dystrophy respectively 0.07)

relation between cell loss and corneal thickness

was found between endothelial cell density and corneal thickness of operated eye ($r = 0.12$ $P > 0.05$ $n = 98$) No correlation was found between the

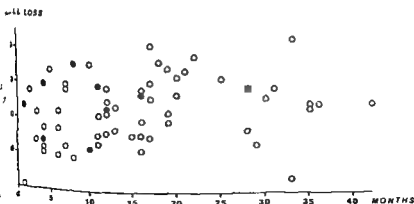


Fig 3

central endothelial cell loss of uncomplicated cases related to time after lens implantation
Meaning of filled and open circles explained in Fig 1

- Olsen T (1979) Non-contact specular microscopy of human corneal endothelium. *Acta ophthalmol (Abh)* 57 986-998
- Olsen T (1980) Corneal thickness and endothelial damage after intracapsular cataract extraction. *Acta ophthalmol (Abh)* 58 424-433
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those patients with or without guttate endothelium. Since the central cell loss also not differ these two groups were pooled and compared to the group with complications. These groups of technical complications, shallow anterior chamber, increased intraocular pressure, all had a significantly higher cell loss than the group of uncomplicated cases. Irrespective of the occurrence of complications, mean cell loss of those with or without progression of dystrophic changes was 15% ($n = 15$) and 53.4% ($n = 9$) respectively (non significant).

In Fig. 3 the central cell loss of uncomplicated cases has been plotted against time after the operation. No significant relation was found. The vertical difference in cell density, i.e. the difference between superior and central counts, was also not significantly related to the post-operative time ($r = -0.21$, $P > 0.05$). The highest densities (about 60% lower density in the superior region) were however found during the first four months. The mean decrease in cell count from the central area to the region 2-3 mm superiorly was 24% (± 20) of uncomplicated cases. No relation was found to the age of the patient in the present series ($r = 0.19$, $P > 0.05$). A somewhat higher difference in vertical density was found in those patients with a guttate endothelium (33.4% vs. 21.5% lower cell count in the superior region for uncomplicated cases with and without dystrophy respectively, $P = 0.07$).

Correlation between cell loss and corneal thickness

No correlation was found between endothelial cell density and corneal thickness of the operated eye ($r = 0.12$, $P > 0.05$, $n = 98$). No correlation was found between the

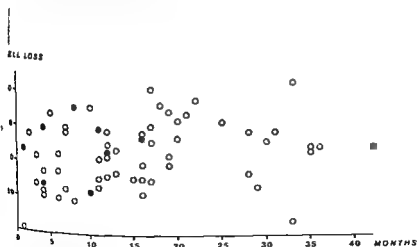


Fig. 3

central endothelial cell loss of uncomplicated cases related to time after lens implantation. Meaning of filled and open circles explained in Fig. 1.

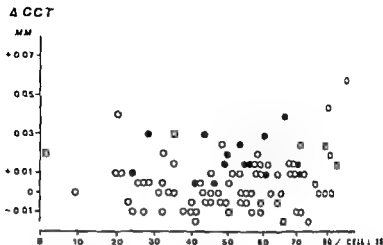


Fig 4

Percentual difference in central endothelial cell count (cell loss) related to difference central corneal thickness (Δ CCT) between operated and non-operated side in 51 eyes with unilateral lens implant. Meaning of filled and open circles explained in Fig 1 ($r = 0.13$ $P = 0.9$)

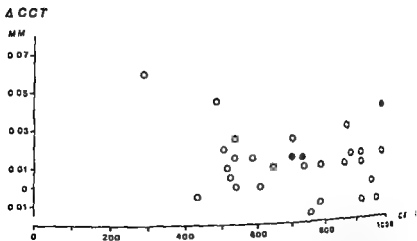


Fig 5

Correlation between residual corneal thickness increase and endothelial cell density in operated eye for cell densities below 1000 cells mm^2 . Meaning of filled and open circles explained in Fig 1 ($r = -0.33$ $P = 0.04$)

loss and residual corneal thickness (Fig. 4). Considering only very low cell densities no significant relationship was found either (Fig. 5). However, when the individual points are considered in Fig. 5 it is seen that two points with a cell density > 500 cells/mm² fell above 0.035 mm, the aforementioned upper normal limit for the residual corneal thickness. One of these patients (cell density 286 cells/mm²) had previously undergone an intracapsular cataract extraction. After the secondary lens implantation she had a short period with a shallow anterior chamber and cornea contact. The operative course of the other patient was uneventful. None of them had endothelial dystrophy or abnormal intraocular pressure.

Discussion

In the present study the most important factor found to influence the corneal thickness after lens implantation was the presence of a guttate endothelium. Progression of dystrophic changes could be said to be the main factor responsible for persistent corneal swelling in six of the eleven patients with abnormal hydration noted in Fig. 1. In the remaining five patients other factors had to be sought.

A remarkable result was that no significant correlation could be established between the endothelial cell loss and the residual corneal thickness increase. For cell densities up to more than 80% or cell densities down to 500 cells/mm² there seemed to be absolutely no association between cell count and corneal thickness. In two patients, however, with a cell density below 500 cells/mm² the cornea was somewhat thicker on the operated side. It cannot be excluded that these two patients thus had a borderline function of the endothelium due to an inadequate number of cells.

Considerations on the prevalence of a low cell density syndrome have to be made with due regard being paid to those corneas in which marked oedema hinders a regular microscopic examination. In two patients with normal endothelium on the non-operated side the endothelium could not be studied due to severe oedema. The oedema in these patients had developed in parallel to the development of ocular hypertension. As indicated in Table III, an increased post-operative intraocular pressure may add to the endothelial cell loss associated with the operation. It is therefore possible that in these two cases the intraocular pressure had caused a further irreversible damage to the endothelium with corneal oedema as a result. In the patient (residual thickness increase 0.04 mm) neither dystrophy, low cell density, or inflammation could be associated with the corneal swelling; the cause of which thus remained uncertain.

The majority of the patients showed a corneal thickness on the operated side which was close to that of the non-operated side. The mean residual thickness

increase of uncomplicated cases without dystrophy of 5 μm although statistically significant — clinically an insignificant swelling and did not change the cause of this swelling remains unknown. A swelling of the same magnitude has been found six months after intracapsular cataract extraction with a cell half of that found in the present study (Olsen 1980).

The higher cell loss in the present study is strongly suggestive of a higher immediate post-operative corneal thickness. While performing the present investigation the corneal thickness of one patient was measured before and the first after lens implantation. An increase of 0.2 mm was found after an untr operation. This is close to the double of the mean increase found in the mentioned study.

The cause of the high cell loss associated with lens implantation needs a consideration. Contact between the lens and the endothelium is one of the factors (Kaufman et al. 1977). The use of an air interface between the cornea and the lens during the operation has been shown to reduce the cell loss (Bourne 1979). The duration of the operation is longer with lens implantation and at that time the endothelium is deprived a normal nutritive supply from the aqueous humour. The composition of the irrigating solution may be of particular importance. Calcium has been shown to be necessary for the integrity of the endothelium (Hajc et al. 1968) and a Ringer solution or other enriched solutions is less damaging to the endothelium than normal saline (Edelhauser et al. 1970).

Whatever the nature of the technical improvements by which the endothelial damage can be reduced, the present study demonstrates that the patient is a factor per se which must not be neglected. One such risk factor is the presence of a diseased endothelium. It is remarkable that the progression of dystrophic endothelial changes occurred independently of the cell density and the surgical cell loss. The provocative factor which caused the progression of the dystrophy therefore remains speculative.

Acknowledgments

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THE EFFECT OF TRANEXAMIC ACID ON SECONDARY HAEMORRHAGE AFTER TRAUMATIC HYPHAEMA

BY

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During the period from March 1978 to November 1979 912 consecutive patients with traumatic hyphaema were allocated by admission-date to conservative treatment and to treatment with the antifibrinolytic drug tranexamic acid.

Secondary haemorrhage occurred in only two out of 107 tranexamic acid treated patients while secondary haemorrhage occurred in 19 out of 80 conservatively treated patients. This difference was statistically significant. Some clinical aspects of the rebleeding cases are presented and briefly discussed.

Key words: traumatic hyphaema, secondary haemorrhage, incidence, severity — prevention by tranexamic acid.

Secondary treated traumatic hyphaema is complicated by secondary haemorrhage (sh) in between 5–6% (Gregersen 1962) and 33% (Crouch & Frenkel 1976) of the cases. Sh usually takes place between day 3 and 7 after the primary trauma. (Gregersen 1962)

Since the secondary bleeding is often more severe than the initial haemorrhage it is often accompanied by increased intraocular pressure and corneal staining. Sh is frequently associated with a poor visual prognosis (Gregersen 1962, Crouch & Frenkel 1976, Pandolfi 1978).

Because of its antifibrinolytic properties the capacity of tranexamic acid in preventing secondary haemorrhage after traumatic hyphaema has been studied during recent years. Favourable results seem to have been achieved in most reports (Crouch & Frenkel 1976, Bramsen 1976, 1977, 1979) although others have failed

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to demonstrate any statistically significant beneficial effect of tranexamic acid therapy (Kamp-Mortensen & Sjølle 1978)

The prospective study of Crouch & Frenkel (1976) includes patients with a cell trait the existence of which might explain the high frequency of bleeding. Nothing however is mentioned in the paper regarding the frequencies of sickle cell trait in the tranexamic acid treated and control treated groups

Bramsens conclusions on the effect of tranexamic acid (Bramsens 1972) are based on combined retrospective and prospective studies with different treatment regimens at various periods in these parts of the study

The material of Kamp-Mortensen & Sjølle (1978) also compares the frequency of bleeding in one group treated with tranexamic acid and one group treated conservatively not investigated simultaneously

Based on the above we found it relevant to perform a purely prospective study on patients with traumatic hyphaema referred to the four eye departments in Copenhagen sharing the casualty ward (Frederiksberg Hospital, Gentofte Hospital, Hvidovre Hospital and Rigshospitalet)

Material and Methods

Material

The material achieved from the four above mentioned departments comprised 232 patients (188 males and 44 females) with an average age of 34.4 years in this study which took place from March 1978 to November 1979. The patients included consecutively

Included into the study were all cases of traumatic hyphaema, which at slit lamp examination showed either a sedimented hyphaema or visible blood in the anterior chamber whereas patients with a haemorrhagic flare only were not accepted for the study. Only patients admitted less than 24 h after ocular injury were included into the study. Other excluding conditions were pre-existing eye disease and perforating eye injuries

Methods

All patients were treated as in patients with closed glaucoma for 7 days. Tranexamic acid was given to patients admitted on even days in a dose of 20 mg/kg body weight three times daily for six days while patients admitted on odd days received neither tranexamic acid tablets nor placebo tablets

The tranexamic acid tablet treatment was initiated as soon as it was possible. No patients had to stop the treatment because of disturbing side effects of tranexamic acid

cut out placebo tablets was considered justified and ethical since the tranexamic acid induced persistence of the primary clot in the anterior chamber and unmask any placebo-blinding.

Allocation of tranexamic acid treatment to admission-date was selected by practical reasons since the material had to be collected from four different hospitals. By the same reason, another statistical method than sequential sampling is to be chosen for calculation of the results (Exact Fischer Test). 95% confidence limits have been employed in the results.

At admission, the primary examination included examination of the visual acuity, evaluation of the anterior chamber, measurement of the intraocular pressure as well as ophthalmoscopy and evaluation of the accompanying lesions. Ophthalmoscopy was not carried out routinely, neither was evaluation of the coagulability of the blood.

The determination of the size of the hyphaema was carried out. On the 5th and 6th day after the trauma, all patients were reexamined.

Results

In the total material, the course was uncomplicated in 94% of the cases, leaving 6% with residual defects.

Table I shows the distribution of the average arithmetic values of the clinical parameters in the tranexamic acid treated group and the conservatively treated group: visual acuity, hyphaema size (in mm) at admission, day and day five after the trauma, age (in years), percentage of males, and length of hospitalization (in days). The mentioned parameters of the two groups are very similar. The only parameter showing a difference is the size of the hyphaema at day five after the trauma. Since tranexamic acid delays absorption of blood from the anterior chamber by maintaining the hyphaema in a clotting stage, the greater average value of the residual hyphaema size at day five after trauma in the tranexamic acid treated group than in the conservatively treated group is understandable.

Table II shows the lesions accompanying the traumatic hyphaemas in the tranexamic acid treated and conservatively treated groups. The severity of the lesions in the two groups, evaluated from the accompanying lesions, is considered equal. Average observation time was 12 days.

Complications

The most important complication was secondary haemorrhage. Out of 107 tranexamic acid treated patients, two patients (2%) developed secondary haemorrhage. These secondary haemorrhages both took place on day three after the trauma, and their course

Table 1

Different parameters in total series and in patients with secondary haemorrhage (a_{SH} plus b_{SH}). Distribution within total series of arithmetic mean values of visual acuity in the tranexamic acid treated group (a) and the conservatively treated group (b). Columns a_{SH} plus b_{SH} distribution of the same parameters in patients with secondary haemorrhage in the tranexamic acid treated group (a_{SH}) and the conservatively treated group (b_{SH}).

	a 102	b 130	a _{SH} 9	b _{SH} 11
Visual acuity day 1 after trauma	0.5	0.6	0.6	0.5
Visual acuity day 5 after trauma	0.9	0.9	1.0	0.9
Hyphaema size day 1 after trauma (mm)	2.0	2.1	1.0	1.1
Hyphaema size day 5 after trauma (mm)	0.3	0.1	0.2	0.1
Age (years)	26.9	20.5	34.3	20.5
Sex (% males)	82.0	60.0	100.0	60.0
Length of Hospitalization Days	6.8	6.5	4.0	6.1
Length of control as out patient (days)	12.0	12.0	12.0	12.0

* Visual acuity at the last control

Numbers on top of columns indicate number of patients in the different groups

was mild without increase in intraocular tension or final visual defects. In these patients did not differ significantly from the whole tranexamic acid group (see Table 1).

Out of 130 conservatively treated patients 12 patients (9%) developed secondary haemorrhage (6 males and one female). These 5 h took place on day two to seven after the trauma with maximum occurrence on day four (five cases). While these cases (see Table 1) again initially did not differ significantly from the whole conservatively treated group in respect to average values of the initial visual acuity, the hyphaema and the sex and age, the final visual acuity in this group was a bit worse (0.7 as compared to 0.9).

Table II

ulated lesions in total series (N 232) Tranexamic acid treated group left column
conservatively treated group right column Numbers in columns indicate percentages

% of patients	Tranexamic acid treated group (N 102)	Conservatively treated group (N 130)
corneal erosion and oedema	28	30
cataract and wound of lids	10	19
intraocular reactions	7	14
traumatic retinal oedema	7	2
transient rise in IOP over 25 mmHg	8	2
dislocation of iris	2	5
retinal and Retinal Haemorrhage	2	4
subluxation of the lens without cataract	1	1
traumatic cataract	2	0
optic nerve atrophy	1	0
corneal hole	1	0
hemochromatosis corneae	0	1
retinal detachment	1	1

Table III

cases and degree of visual impairment in cases of rebleeding in the group conservatively
treated patients with traumatic hyphema For further discussion see text

glaucoma treated with anterior chamber evacuation	1 case (vision less than 6/1)
right traumatic cataract	
glaucoma and persistent anterior chamber haemorrhage Psychosis	1 case (vision light perception)
corneal haemochromatosis following glaucoma	1 case
right traumatic cataract	(vision 0/1)
glaucoma with transient corneal staining persistent anterior haemorrhage psychic reaction	1 case (vision hand movements)
transient glaucoma and recession angle	1 case
choroidal rupture and transient corneal staining	(vision 3/12)
traumatic cataract	1 case (vision 6/9)

The average length of control for the 12 cases of the control group was 60 days compared to 19 days for the conservatively treated group. The average period of hospitalization for the same 12 cases was 15 days. The average length of hospitalization for the whole conservatively treated group was only 6.5 days. In the conservatively treated group the 5 h were only mild with none to normal in six out of the 12 cases mentioned. The remaining six cases were severe course are presented in Table III. The five of these six cases had increased intraocular tension above 50 mmHg for more than three days. Two out of the six favourable cases developed a rise in intraocular tension to 55 mmHg for 1-3 days. Additional remarks on complications

Although not included into this study because of the criteria for inclusion in the study nine patients were admitted to hospital during the study period with a 5 h after trauma. Eight patients presented with 5 h on day two to four after trauma, and one patient presented secondary vitreous haemorrhage six days after an unrecorded trauma. One period one patient was admitted with a spontaneous haemorrhage in the anterior chamber.

Discussion

Although only occurring in the minority of the cases of traumatic hyphema (Gregersen 1962) 5 h should be prevented by the above mentioned measures. Antifibrinolytic drugs have been suggested and tried in hyphema treatment based both on their penetration into the aqueous humour and on their haemostatic employment since 1968 in the treatment of a number of systemic haemorrhagic conditions associated with hyperfibrinolysis (Crouch & French 1970, Foulds 1978).

Since the possible hyphema treatment results referred to in the literature are calculated from combined prospective/retrospective materials and degrees of physical activities in the different parts of the study, (Björnskov 1977, 1979) and from materials including patients with sickle cell trait (French & Frenkel 1976) these results all seem to be based on patient material not equivalent to the present material. In the present study on 232 white patients who were all amblyopes treated with bed rest and stenopaeic glasses roughly half the patients in addition received cyclopropanol as a dose of 75 mg/kg body weight orally daily for 14 days. Reduction statistically significant at the 2% level (Exact Fisher Test) was demonstrated in the incidence of 5 h in the traumatic and treated group.

the mild course of the two s.h. in the tranexamic acid treated group corresponds to similar observations made by Crouch & Frenkel (1976).
 The severe course of the six out of the 12 cases of s.h. occurring in the conservatively treated group is in agreement with findings made by Gregersen (1977) who reported increased intraocular tension with corneal staining and recent bleeding in five of 11 conservatively treated patients with s.h. Crouch & Frenkel (1976) reported a need of clot removal due to increased intraocular pressure and corneal staining in two out of nine conservatively treated patients with s.h. All three cases of s.h. reported by Kamp-Mortensen & Sjølie (1978) were of a mild course without increased intraocular pressure or corneal staining.
 Based on the outcome of the present study a new prospective study had been initiated, aiming to evaluate the safety of tranexamic acid therapy alone without restricted physical activities in the future therapy of traumatic hyphaema. In this study all the patients henceforward will be treated as in patients with tranexamic acid. With further random division of the patients participating in the study into those treated with bed rest and those treated out of bed.

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THE USE OF CONTACT LENSES IN WET OR DAMP ENVIRONMENTS

BY

PER LÖVSUND SVEN ERIK G NILSSON
and P ÅKE ÖBERG

The adhesion between the eye and a contact lens was recorded when influenced by water with varying salt concentrations. Both active removal and spontaneous loss of hard and soft contact lenses applied to the eye were investigated.

In environments with saline $< 9.0/00$ the risk of a soft contact lens falling out from the eye is almost negligible. In environments with saline $\geq 9.0/00$ the risk increases since adhesion in active removal decreased considerably. Is based upon the investigations of spontaneous loss of contact lenses at an underwater environment, well fitted soft lenses still do not however fall out of the eye. It should therefore be possible to use well fitted soft contact lenses without any significant risk even in working conditions which may involve splashing of water upon the eye and regardless of the various salt concentrations of naturally occurring water in Sweden.

For hard contact lenses independent of salt concentration adhesion at the underwater environment is extremely low and such lenses cannot therefore be used on an unprotected eye in conjunction with, for example, swimming. The risk of them falling out under splashing with water upon the eye is probably greater than for soft lenses.

Key words: contact lenses - adhesion - wet environment - water sports hazards - worker's safety

The considerable increase in the use of contact lenses had made it necessary to analyse the risks and advantages of such lenses under various working conditions and in various sports. Previously animal experiments have been carried out

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investigate danger in conjunction with electric welding (Lovsund et al. 1979a) and infrared heaters (Lovsund et al. 1979b).

The question of the use of contact lenses in wet and damp environments is unresolved. The problem is seldom discussed in reference literature although the risk of losing both hard and soft lenses in conjunction with water sports have been assessed (Hales 1978). The possible risk for personnel on fast, open customs cruisers of losing contact lenses by heavy splashing with water upon the eyes has been discussed extensively among authorities and at present such employees in Sweden are not permitted to use contact lenses when on duty. A couple of practical experiments with contact lens users on boats have been conducted but these have been of such a type and scope to enable any definite conclusions to be drawn. The question is therefore still unsolved. Since it also involves a number of other working areas and sports in wet or damp environments it is important that scientific studies be carried out to provide a firmer basis for establishing for example regulations concerning requirements for employment and exemption granting.

In this study adhesion between the eye and a contact lens has been recorded when affected by water with varying salt concentrations. The project has comprised investigations of I active removal and II spontaneous loss of contact lenses applied to the eye. Both hard and soft (low and high water content) lenses have been investigated.

Material and Methods

I Active removal of contact lenses applied to the eye

When recording the adhesion between a contact lens and the corneal epithelium used contact lenses with a suture thread glued on to the centre of the lens. The lens was pulled away from the eye using an electric motor and the tensile force was registered. The force at the moment at which the contact lens separated from the eye was taken as measure of the adhesion.

Table I
Contact lenses used

Study No	Lens-make	Material	Water content (%)	D opter	Diameter (mm)
III	Softlens	HEMA	39 (soft)	approx +1.5	13.6
III	Scanlens	PMMA	approx 1.2 (hard)	approx -0.5	9.6
II	Softlens	HEMA	39 (soft)	approx -0.5	13.6
II	Scanlens	HEMA	80 (soft)	approx +1.5	13.0

The investigation involved five volunteers (two men and three women), one regularly wore contact lenses. No difference appeared between the regular user of contact lenses and the subjects who did not use them. The contact lenses which were individually fitted by a contact lens technician were of two types: hard lenses and soft lenses with low water content (Table 1). The volunteers were given Novesin® 0.4% eye drops as topical anaesthesia, which made it possible for the test subject to keep his eyes open and to reduce eyelid pressure to a minimum. Between the various experiments the lenses were cleaned and disinfected in the prescribed way. A 0.09 mm suture thread (Ethicon® 6/0 707G) was glued in the centre of the front of the lenses using a silicon glue (Medical Adhesive Silicone Type A, Dow Corning). The glue was applied as uniformly as possible to the different lenses and the least possible amount was used in order to minimize deformation of the lens. The glue could not be made

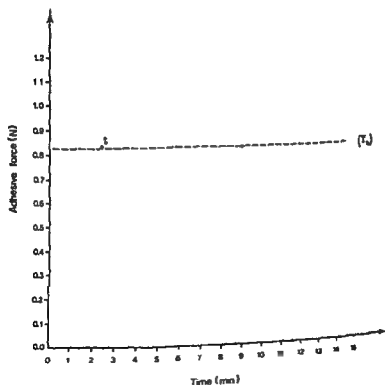


Fig. 1

All recordings of adhesive force between cornea and contact lens and the period of active removal of soft contact lenses when affected by water with salt concentration of 1.7 and 8.0 00 or in air (5.5 pull distributed among 5.5 subjects).

were sufficiently firmly to high water content soft contact lenses and these are therefore excluded from this part of the investigation.

From the contact lens the suture thread went to a force transducer from which a suture thread was attached to the shaft of a motor. The force transducer (MicroMed Limited) consisted of an unbonded strain gauge element with a maximum load range of ± 2.27 N. The force transducer was bridge coupled and the signal amplified (AC Carrier Preamplifier HP) and registered on a recorder (Cathode ray chart recorder HP). A DC motor (Escap) fitted with a gear box giving a reduction of 20:1 was used to pull the contact lens from the eye. A constant speed (0.6 mm/second) was maintained during the entire pulling cycle. In order to expose the eye and the contact lens to water with various salt concentrations a plastic film intended for covering around a surgical operation area (Drape[®] 3M) was used. The film had a central hole 63 mm in diameter.

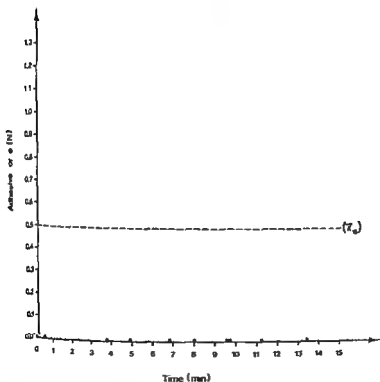


Fig. 2

Recordings of adhesive force between cornea and contact lens and the mean of these in the removal of soft contact lenses when affected by water with a salt concentration of 9.0.00 (40 pulls distributed among 20 volunteers)

surrounded by an area with an adhesive coating which allowed the film to be attached to the skin around the eye of the supine volunteer. The edges of the film were then folded up and attached to a ring placed above the head of the subject. This arrangement provided a watertight receptacle for the various salt solutions. The receptacle was filled to the same level each time.

The salt solutions were prepared from distilled water and NaCl and were kept at room temperature (23–24 °C) during the experiments. Recordings were made at salt concentrations of 0.1, 1, 7, 8, 9, 10, 11, 13 and 25.0/00 and also on the removal of any liquid other than normal tear flow.

The lens was always applied to the right eye and in the same way every time. Air bubbles were allowed between the eye and the lens and it was checked that the lens really was attached to the eye. When the salt solution was poured into the plastic receptacle the pulling could start. After the lens was pulled away the solution was removed and the lens could be applied to the eye again. The registrations were continued during an effective time of 15 min in each solution.

11 Spontaneous loss of contact lenses applied to the eye

In order to study whether or not contact lenses spontaneously fall out from the eye in the underwater environment without any tensile force the volunteers wore their faces in the various salt solutions for a period of maximum 15 min. They were instructed to blink frequently and in between blinking to open the eye and to perform eye movements in various directions. If the lens fell out of the eye the time when this occurred was recorded. The volunteers used snorkels for breathing. The study comprised the same volunteers as in Test I. Apart from the contact lenses used in Test I soft, high water content lenses were also used (Table 1).

Results

1 Active removal of contact lenses applied to the eye

Soft, low water content lenses

With regard to the force necessary to pull away the lenses from the eye no significant differences ($P \leq 0.05$) were recorded for the various salt concentrations $\leq 8.0/00$ (0.1, 1, 7 and 8.0/00) either among themselves or between the values in air. All these measurements are therefore shown in Fig. 1. At 9–10.0/00 the mean value for adhesive force began to fall at the same time as the individual measurements showed a large dispersion (Figs. 2 and 3). At salt concentrations $\geq 11.0/00$ (11, 13 and 25.0/00) adhesion was very low and without any significant differences.

0.05) among themselves (Fig. 4). A summary of the results for all salt concentrations is shown in Fig. 5. In order to clarify further the relationship between salt concentration and adhesion a number of experiments were carried out in which the salt concentration was first increased in steps of 1.0/0.0 from 7 to 11.0/0.0 and then decreased directly to 0.0. A typical experiment from this series is shown in Fig. 6. With regard to the different concentrations the adhesion picture generally agrees well with the results in the entire group of volunteers (Figs. 1-5). At 7 and 8.0/0.0 the values are high at a high level. At 9 and 10.0/0.0 the dispersion in the measurements is large, the mean is clearly lower. At 11.0/0.0 the mean falls further. Upon a return to 7.0/0.0 the adhesion value rises to a level which corresponds approximately to the value at the beginning in 7.0/0.0 concentration.

2
lenses

3
adhesive force for hard lenses was considerably lower than for soft lenses. In measurements in air i.e. with normal tear flow over the eye adhesion was recorded

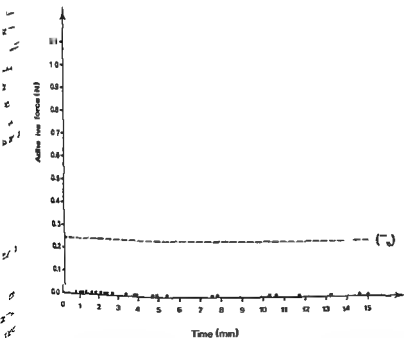


Fig. 3

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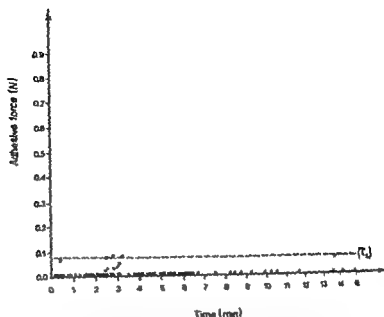


Fig. 4

All recordings of adhesive force between cornea and contact lens and the mean of active removal of soft contact lenses when affected by water with salt concentrations of 0.19 and 0.00 (100 pulls distributed among 20 volunteers).

as 0.09 ± 0.01 N. In tests with water with various salt concentrations adhesive force was extremely low (< 0.01 N) independent of the salt concentration.

II Spontaneous loss of contact lenses applied to the eye

Soft low and high water content lenses

Not a single lens fell out during the 15 min test period despite frequent blinking and eye movements. The salt concentrations were 0.19 and 0.00. At a concentration of 0.19, the lens on occasion adhered so firmly to the eye that it could not be removed until after dropping 9.000 saline for about 5 min. Five trials per lens type at each concentration for each of the five volunteers.

Hard lenses

All the lenses fell out at all salt concentrations (0.1, 0.3, 0.5, 0.8, 0.9 and 2.0) at a mean time of about 1 min. No definite difference in mean time was observed between the various salt concentrations. Four trials at each concentration for each of the five volunteers.

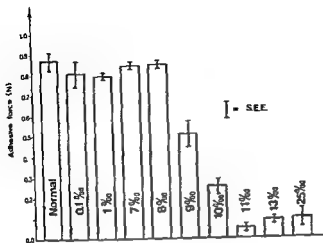


Fig 5

mean of the adhesive force between cornea and soft contact lenses when affected by varying salt concentration or in air (normal) (20 volunteers) SEE = standard error of the estimate

Discussion

enses

study shows that salt concentration exerts a clear influence on the adhesion between soft contact lenses and the eye. From firm adhesion at salt concentrations of 0.00 the values became highly variable at 9 and 10.00 while the adhesion was throughout considerably lower at salt concentrations ≥ 11.00 . The change in adhesion seems, however, to have not so much practical importance as was at first sight, since the soft lenses did not fall out spontaneously from the eye in the treatments in underwater environment (Test II) which simulate real life better. Lenses did not fall out even after vigorous and frequent blinking and eye movements. It should however be emphasized that the experiments were carried out using well fitted lenses. There would appear to be a substantial risk of less tightly fitted lenses falling out of the eye in water with a salt concentration ≥ 11 . The same risk may arise in conjunction with certain pathologically changed eyes, e.g. in keratoconus. In these cases however hard contact lenses are usually used. The question arose as to whether prolonged exposure to water alone, irrespective of salt concentration, could change the corneal surface, e.g. by washing away its mucus, so as to prevent adhesion of a contact lens. The experiment shown in Fig 6

Acknowledgements

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SPHEROID DEGENERATION OF CONJUNCTIVA AND CORNEA

Two Years Follow Up

BY

MOGENS NORN

Twenty six subjects with spheroid degeneration were followed up after two years. The number of colourless conjunctival droplets was seen to have increased by on an average 46% ($2\alpha = 0.05$) and that of autofluorescent conjunctival droplets by 223% ($2\alpha < 0.01$).

Counting within the individual sites disclosed that some droplets will disappear (not less than 30 and 21% respectively) while recently formed will consist at least 16 and 243%.

The number of areas with band shaped keratopathy was found to rise to 26 ($P < 0.001$) out of 104 possibilities (nasally and temporally of each left eye).

Vital staining (fluorescein rose bengal tetrazolium alcian blue) showed epithelium above the droplets to be intact and the droplet-containing mucus were found not to be abnormally dry (break up time tear production).

Keywords: degeneration spheroid - keratopathy bandshaped - conjunctiva cornea - break up time

Spheroid degeneration of cornea and conjunctiva implies the presence of ball shaped elements in the exposed region concerned. Such droplets range in size from small as to only just be visible under $\times 10$ magnification with the Haag Streit slit lamp up to 400 μm . They are generally limpid more rarely yellowish and autofluorescent. Under direct lighting the distant half of the droplet is luminous while the proximate half is shaded owing to the refractive index of the droplet compared with that of its surroundings.

The droplets may be so numerous on the cornea as to form bands along the axis, or possibly across the cornea. The phenomenon is then called Bietti's nodular dystrophy climatic droplet keratopathy or Labrador keratopathy.

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In a previous study (Norn 1978) the author noticed that spheroid degeneration was most frequent on the conjunctiva. These patients have been summoned for follow up examination to see whether such droplets increase in number. It seemed to be the case, the prevalence rising with increasing age, but not, however, after the age of 70.

Material and Methods

The initial series comprised 33 patients with spheroid degeneration found in 810 Copenhageners.

Of these 26 were summoned to a follow up examination two years later (two died and five could not be traced). They all attended the examination in morning at the eye department.

The examiner was the same in all cases and proceeded in the same manner as at the initial examination (see Norn 1978).

In addition the eyes were examined for precorneal fatty layer by the interferometry method (Norn 1979b). BUT (break up time) tear secretion (lacrimal meniscus test) and by means of different vital stains mentioned in succession: fluorescein, rose bengal, tetrazolium and alcian blue mixture and possibly alizarin (Methods see Norn 1974). The results were compared with those of corresponding examinations of the contralateral normal eye.

Table I

	Initially	Follow up		
		Mean	Increased	Reduced
<i>Conjunctiva</i>				
Limpid	645	943*	483	193
Autoflu	53	171**	199	11
<i>Cornea</i>				
Microcysts (ca.)	180	50.5	48.7	0
Limpid	9	11	11	9
Autoflu	3	7	5	1

Change in number of droplets after two years. The figures indicate the number of droplets per subject (26 subjects).

* $2\alpha = 0.05$ ** $2\alpha < 0.01$

Table II

	Initially	Follow up		
		Mean	Increased	Reduced
Conjunctiva				
Lipid	44	56	15	3
Autoflu.	31	39	15	-
Cornea				
Lipid (ca.)	3	26	23	11
Lipid	2	2	9	9
Autoflu.	2	3	9	1

ber of sites with spheroid degeneration. A total of 104 sites (nasally and temporally of right and left eye, four areas per subject, 26 subjects examined)

0.001

Results

Conjunctiva

number of colourless droplets rose by 46% in the course of two years. The rise was significant (non-parametric Mann-Whitney rank sum test $2\alpha = 0.05$).

The number of autofluorescent droplets rose by 223%. This rise was likewise significant ($2\alpha < 0.01$).

The mean values have been set out in Tables I and III.

Each of the 26 subjects had four areas examined (nasally and temporally of right and left eye) a total of 104 sites. The proportion of sites showing droplets increased in two years, but the difference is not significant (Table II). In some instances droplets disappeared from one area, while new ones might appear in a hitherto droplet-free region.

Table III

	Initially	Follow up
Conjunctiva		
Lipid	24.8 ± 9.0	36.3 ± 8.4
Autoflu.	20 ± 0.4	66 ± 2.4
Total	26.8 ± 9.4	49.8 ± 9.4

Change in number of conjunctival droplets after two years. Mean values (\pm SEM) per subject, a total of 26 subjects.

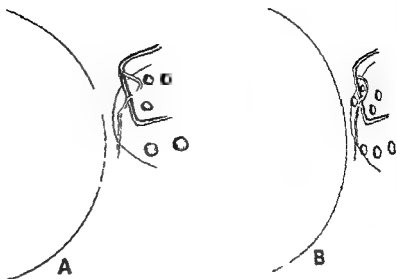


Fig 1A

Initial examination of case with four autofluorescent (hatched) and one limpid droplet
pinguecula

Fig 1B

Shows the same site two years later. The two autofluorescent droplets within the bend
large vessel have disappeared. The two others still exist below the vessel and a new one
appeared closer to the limbus corneae. A total of three autofluorescent and four
droplets

Table I (two right columns) illustrates 1) the numbers of droplets noticed in
areas and 2) areas from which previously present droplets had disappeared.

In one case droplets were mapped out within one region by means of the
of some blood vessels. Fig 1 illustrates the result.

The variations were greater than the calculations in Table I indicate.
droplets occurred within the same area while old ones disappeared. Clear
droplets became autofluorescent while some fluorescent droplets were seen
this property. The variations were independent of occupation or retirement.

Cornea

Limpid and autofluorescent droplets were present in few cases only. Such
increase significantly in number (Tables I and II).

Band shaped keratopathies consisting of fine microspheric granules
significantly in number (Table II, t test $P < 0.001$) from three in two patients
in nine patients. These bands were all localized vertically along the limbus.

Table IV

	With spheroid	Control eye
Precorneal fat (nm)	114	124
Break up time (sec)	23.0	20.4
Tear production (dilution/2 min)	261	205
Rose bengal vital staining score*	3.9	2.3

Surface phenomena in 40 eyes with conjunctival spheroid degeneration compared with the conditions in 12 contralateral eyes (mean values)

* maximum 15 per eye

not yet grown towards the centre representing in other words grade 1 erosion according to Freedman's classification (Freedman 1967)

The corneal droplets were most often localized within areas showing micro-erosions (in four out of six cases)

As stated above the droplets differed in size (from 30 to 400 μ m). There was one exception however who presented a 3 mm long streak along the limbus conjunctivae. The lower one third of this was autofluorescent while the upper two-thirds were clumped constituting presumably a conglomeration of many droplets.

Surface Phenomena

As shown in Table IV the fatty layer covering the precorneal film and the stability of the film (BUT) proved to be independent of the presence of spheroid degeneration of the conjunctiva and the mean values did not differ from those of a normal series.

The tear production was perhaps slightly reduced in eyes with droplets and the instability by rose bengal in the exposed part of the eye correspondingly increased. However the difference from the control eye was not significant and the results were within the normal range.

Vital Staining

Rose bengal did not specially stain the epithelial regions covering the droplets. On the other hand some pingueculae were stained diffusely (43% out of 80 pingueculae) particularly such as were elevated. Vital staining by fluorescein (reach of epithelial continuity), tetrazolium (degenerate enzyme holding epithelial cells) and blue (mucus) and alizarin red (calcium) did not differ from the conditions in a normal series. In particular these dyes did not specially affect the droplets.

Occupation

Two-thirds of the subjects had mainly outdoor work (sailor, gardener, painter, navy, surveyor, cemetery attendant) and one third indoor work (office, cleaning, nursing).

Discussion

Spheroid degeneration was first detected on the cornea. Frauenfelder et al (1977) also noticed such droplets on the conjunctiva. Garner et al (1976) and Vorn (1979) found the conjunctival site to be much more frequent than the corneal site.

The pathogenesis is obscure. Corneal spheroid degeneration is most frequent in high geographic regions having an intense ultraviolet light (the Red Sea, Labrador).

The following facts go to show that conjunctival droplets also are provoked by ultraviolet light. The geographic difference in prevalence between Greenland and Copenhagen (Vorn 1978), the high incidence of pingueculae (Vorn 1979a) which likewise is supposed to be caused by ultraviolet light, and the predominance of outdoor occupation, even though the present screening material comes from a city.

The droplets are localized within exposed areas, both on the conjunctiva and on the cornea. Dessication might therefore be conceived to be a contributory cause.

However, the results of the present investigation (BUT vital staining) argue against this theory.

The follow up showed the number of droplets to have increased rapidly within two years. Some droplets had disappeared within the same period. Thus we get an impression of a variegated picture with development of many new droplets. Large droplets become yellow and autofluorescent while others lose their colour and some even disappear completely.

This disappearance was a quite new and unexpected observation. The possibility might be conceived of a loss through the epithelium. However, vital staining affords no evidence to support this view (no epithelial cell damage, breach of continuity nor mucus coating). The refractive index of the droplets may perhaps alter to be equalized with the surroundings or the droplets may become so much reduced in size as to become unrecognizable ($< 30 \mu\text{m}$).

The prognosis of conjunctival spheroid degeneration is usually good, the affected being harmless with no subjective complaints. Yet persons with droplets on the conjunctiva are subsequently liable to develop band shaped keratopathy and presumably in most cases concentrated peripherally. In few cases only will the droplets spread across the cornea to form the rare type III with considerable impairment of the central vision.

In the presence of band shaped keratopathy the finding of droplets in the conjunctiva is of value in the differential diagnosis. In cases with band shaped areolar deposits decalcification is obtainable by tetracemine disodium (EDTA) treatment has no effect on band shaped spheroid degeneration. We have started electronmicroscopic examinations of conjunctival droplets and will follow up examinations of the biopsy site.

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GALACTOSAEMIA WITH CATARACT AND PERSISTENT HYALOID ARTERY A Clinicopathological Case Report

BY

P VANGSTED

A boy with galactosaemia and bilateral cataract developed large intraocular haemorrhages in the left eye which was enucleated.

Histologically a persistent hyaloid artery was demonstrated. This combination with galactosaemia seems not to have been described in the literature before. The haemorrhages are most likely secondary to the persistent hyaloid artery system. In addition some foci of extramedullary haemopoiesis were demonstrated in the retina.

Key words: galactosaemia – arteria hyaloides persistens – retinal extramedullary haemopoiesis

We have recently investigated a case of galactosaemia with some peculiar findings which appear not to have been described before.

Clinical History

The patient was a 10 month-old boy, the third child in a family without known congenital (inborn) errors of metabolism or other malformations. In 1968 the mother bore a premature girl, weight 1200 g, who died after 94 h (no autopsy). In 1969 during the last month of her second pregnancy the mother had slight hypertension but the delivery

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Table I
Laboratory findings at first examination

	Findings	Normal values
tose	310 mg/100 ml	0
tose (Thin layer chromatography)	+++	0
tophan (Thin layer chromatography)	+	0
sine (Thin layer chromatography)	+	0
smuna	+	0
tose 1 phosphate	658 µgr/ml eryth	0
tose 1 phosphate undyl transferase	2.5 µmol LDPG pr h Hb	27.6 ± 3.7 (X ± SD)
line phosphatase	1700	13-38
phosphatase	28	2-10
ine amino-transferase	90	5-25

ere normal. Examination of the mother revealed monolateral squint with a bit malformations. During the last trimester of the recent pregnancy the mother lost weight. Uncomplicated delivery in November 1977 in due time; length 49 cm. The boy did not thrive and after a few months the mother noticed a non-reacting left pupil. In April 1978 the 5-month-old child was referred to the Department of the Rigshospital because of suspected retinoblastoma. A diagnosis of galactosaemia was established and the child was put on a galactose-free diet. Immediate improvement of the clinical condition and the abnormal laboratory findings was found and among others the following laboratory examinations were performed:

Ophthalmological Findings

Right eye
No strabismus. corneal diameter 11 mm. slight posterior cortical and nuclear cataract. Behind the lens a persistent tunica vasculosa was noticed but normal fundus and no persistent hyaloid artery. Electroretinogram (ERG) and ocular ultrasound normal.

Left eye
corneal diameter 10.5 mm. flat anterior chamber and a dilated pupil with pronounced ectropion uveae simulating partial aniridia. A small lens with anterior subcapsular cataract, nuclear and posterior cortical cataractous changes. A retrolental

white to yellowish brown membrane was noticed transillumination revealing shadow downwards. The ERG was extinct tension slightly increased. Ultrason examination supported the diagnosis of congenital membrane anteriorly & vitreal haemorrhages.

Five months later the cataract in the right eye had diminished and the neovasclosa had disappeared. The left eye was blind the lens was now subluxated downwards still cataractous and a massive grey black membrane was now behind the lens. Ultrasonic examination (A & B scanning) now suggested a lesion in the posterior half of the blind eye which was enucleated.

Ocular Pathology

Macroscopical examination (Eye Path Inst no 662/78)

The eye measured 21×19×19 mm the corneal diameter 10×10 mm. The eye was maximally dilated. Vertical sectioning the lens was cataractous and 1 haemorrhages were seen in the upper half of the eye. Inferiorly a greenish disc. The disc and the short optic nerve were punched out and cut with 10 serial sections.



Fig. 1

Eye Path Inst No 662/78 Extreme proliferation of iris pigment epithelium coming anteriorly and invading the chamber angle (arrow). Connective tissue in the anterior chamber (large arrow). Haematoxylin-eosin stain (x 110).

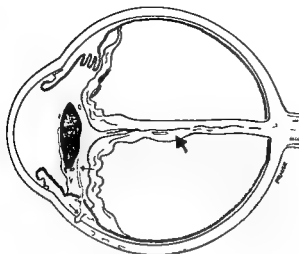


Fig 2

Schematic drawing of the sagittal section of the enucleated eye. Totally detached retina (large wavy line). Inferiorly the persistent hyaloid artery anastomoses with an artery in the ciliary body and sclera.



Fig 3

Persistent hyaloid artery on its way to the anastomosis in the ciliary body (arrows).
Haematoxylin-phloxine-saffranine stain ($\times 98$)



Fig 4

Extra medullary hemopoiesis in the retina (arrow) (Haematoxylin eosin stain (X 1000))

anterior to and in the area of the lamina cribrosa. About 220 sections of the eye were cut including some sections of both calottes.

Microscopical examination

Slightly diminished eye with dome shaped cornea. The anterior chamber is shallow centrally due to posterior subluxation of the small lens. Peripherally the chamber is flat due to an enormous ectropion of the iris with anterior synechia to the cornea. The iris pigment epithelium continues on the anterior surface of the iris (Fig 1). The chamber angle is filled with fibrovascular tissue from the iris and some iron positive macrophages and lymphocytes are noticed. Schlemm's canal is present but partly occluded. There is slight capsular cataract anteriorly and posteriorly. The lens epithelium is partly two-layered anteriorly and covers the whole cortex including the posterior part where a few bladder cells (Weber) are seen.

The lens is completely surrounded by a fibrovascular membrane with retrolental of haemorrhages. The vitreous is small, triangular and contains a large peripheral hyaloid artery which anastomoses with an artery in the ciliary body inferiorly (Fig 2) and 3). The hyaloid artery can be traced back into the middle of the funnel-

ly detached retina. Superiorly an oral retinal tear is noticed in some sections retina is degenerated with enormous gliosis and some foci of extramedullary opoiesis are seen (Fig. 4). Behind the detached retina there are pigment telum membranes and old haemorrhages. Ciliary epithelial proliferation and a ounced Ringschwiele are noticed. Iron stain is positive in the retina and lens telum. Alcan blue, Colloidal iron and P.A.S. stainings reveal no abnormal xaminoglycans in the cornea or sclera when compared with two other eyes children with primary hyperplastic persistent vitreous. Murexid staining for am is negative. No fungi or bacteria in special stainings. Transversal sections of isc and the atrophic optic nerve reveal normal central vessels without thrombi.

Comments

high excretion of galactose in the urine and the very elevated galactose 1 phosphate of the erythrocytes together with the very decreased concentration of roctic galactose 1 phosphate-uridyl transferase confirm the diagnosis heredi galactosaemia. The treatment resulted in regression of symptoms. In galacto- ia, a few days after birth galactose 1 phosphate accumulates in the lens and etabolism becomes disturbed. Untreated the disease results in cataract al retardation and hepatic damage followed by cirrhosis. In minor galactosea cataract can be the only clinical manifestation (Christini 1957, Torselli & lena 1960). In a single case of galactosaemia cataract has been demonstrated etron microscopy in a five month old fetus (Vannas et al. 1977). At the age of 17 weeks the cataract often appears as a ring resembling a drop of oil in the e of the lens (Nordmann 1966). About 2 weeks later it begins to spread into al vacuoles and progresses to complete opacification of the lens.

re literature from 1908-53 has been summarized by Patz, who found cataract as olv ophthalmological complication to galactosaemia. Wilson & Donnel (1958) d eight cataracts in 12 patients with galactosaemia but no other ophthalmologi mplications. Hsia & Walker (1961) found two cases of galactosaemia with ocular haemorrhages and detachment of retina in one case resulting in loss.

o my knowledge a persistent hyaloid artery has not previously been described in exion with galactosaemia.

our case there are two possible explanations: 1) A persistent arteria hyaloidea m with large intraocular haemorrhages after birth. 2) Intraocular haemorrha e before or during the 7th embryonal month causing persistence of the hyaloid ena.

The first possibility fits well with the ultrasonic findings and seems the probable in this case

An interesting finding in this case is that the persistent hyaloid artery anastomoses with an artery in the ciliary body and the episclera

Some foci of extramedullary haemopoiesis seen in the retina are also discovery The hemopoietical tissue of mesodermal origin is normally seen in the vascular plexes around the optic cup (Barber 1955)

A rupture of these vessels followed by migration of the cells towards the surface may explain this finding

Follow up April 1980

The boy is still on galactose free diet, and his condition and visual acuity improved

The boy is now able to collect small balls from the floor and follows with both subjects with the remaining eye

There is still an undulatory nystagmus slight zonular cataract and posterior polar cataract

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ADVANCED RETINOBLASTOMA IN MALAYSIAN CHILDREN

BY

D. SINNIAH, G. NARASIMHA and K. PRATHAP

Twenty children with retinoblastoma are reviewed who were treated at the University Hospital Kuala Lumpur over a 10-year period. They constitute 6.6% of childhood malignancies and without exception all presented with advanced disease. Hereditary cases were notably absent in the series probably because past cases have almost invariably succumbed without an opportunity to transmit the gene. With enucleation and radiotherapy six of the patients have survived from 2 to 12 years. The addition of vincristine and cyclophosphamide has not been associated with improved survival.

Keywords: retinoblastoma — histological staging — therapy — survival

Retinoblastoma, the commonest intraocular tumour in childhood, occurs either sporadically as a result of spontaneous mutation or as an inherited autosomal dominant characteristic. Its frequency varies from 1 in 15 000 to 1 in 30 000 live births (Schappert, Kimmijser et al. 1966; Francois & Van Leuven 1964). An increasing incidence has been recorded in recent years and this has been attributed to improved methods of treatment resulting in more survivors transmitting the genetic defect and to an increase in the rate of spontaneous mutation (Bedford & Zimar 1975). The paucity of data from developing countries has prompted us to review the epidemiological, clinical and histopathological features, the staging and response to treatment in children with retinoblastoma seen at the University Hospital, Kuala Lumpur, over a ten year period as compared with those from other countries.

Received January 8, 1980

Materials and Methods

All cases of retinoblastoma admitted to the Paediatric Unit during the period 1 through 1977 were studied. Details of the illness, family history, investigation, response to therapy and outcome were reviewed. The tumours were staged according to the scheme proposed by Reese & Ellsworth (1963) and modified by Bedford et al (1971). Since 1976 stage V and VI cases have received all 3 modalities of treatment, namely surgery, radiotherapy and chemotherapy. The latter is: vincristine 1.5 mg/M² IV plus cyclophosphamide 300 mg/M² IV once every 4 weeks for one year. Eyes enucleated as part of treatment were subjected to detailed histopathological review to confirm the diagnosis and staging and to study the relationship to prognosis.

Results

Epidemiology

During the index period 20 children (9 males and 11 females) with retinoblastoma were admitted and they account for 6.6% of all childhood malignancies. They comprised 13 Chinese, 4 Malays and 3 Indians who reflect the racial composition of patients admitted to the Unit. Their mean age at presentation was 30.7 months, ranged from 8 months to 7 years. 14 were aged less than 3 years. Unlike others (Bedford et al 1971) there was no family history of retinoblastoma although 2 children presented with bilateral disease.

Clinical Features

The mean duration of symptoms prior to diagnosis was 8.4 months and ranged from 3 days to 30 months. The commonest symptoms at the time of diagnosis were white pupil reflex, loss of vision and swollen, painful red eye. None of the patients complained of squint as seen in Table 1.

The tumour originated in the right eye in 13 cases, the left eye in 5 cases, bilaterally in 3 cases and was unrecorded in 1 case respectively. Six children

Table 1
Clinical features of 20 cases of retinoblastoma

Presenting Symptoms	No. of cases
White pupil reflex	17
Loss of vision	3
Swollen painful red eye	3
One pupil bigger than the other	1
Squint	0

of V disease 12 had stage VI disease and in 11 patients the stage was not recorded. The VFA screening test was positive in 5 of 8 cases. Chest X ray and skeletal survey revealed orbital calcification in 3 cases with blurring of the orbital margin in 1 instance. Cerebrospinal fluid yielded malignant cells in 2 cases and evidence of malignant meningitis in another.

Treatment and Outcome

Of all the patients presented with either stage V or VI disease. Their histological features, treatment and outcome are recorded in Table II. Two patients refused treatment while the rest had enucleation of the affected eye. Of 10 patients who had only enucleation done 2 were lost to follow up, one died of post-operative meningitis while 2 others are tumour free 4 and 6 years after surgery respectively. Ten patients received post-operative radiotherapy and of these have survived from 2 to 10 years without tumour recurrence. 3 were lost to follow up and 2 developed widespread metastases.

Three patients with stage VI disease received all three modalities of treatment: enucleation, surgery, radiotherapy and chemotherapy. One patient (case No. 16) developed CNS infiltration with cerebrospinal fluid involvement and was successfully treated with intrathecal methotrexate and systemic vincristine, adriamycin and cyclophosphamide (VAC) but was lost to follow up. The other 2 (cases No. 17 and 18) suffered from re-emergence of tumour whilst receiving continuation chemotherapy and of these one was lost to follow up while the other succumbed to metastases despite intrathecal methotrexate and systemic (VAC) therapy.

Discussion

Until now no studies on retinoblastoma have emerged from this part of the world. Its incidence has not been established in Malaysia but it accounts for 0.6% of all malignancies in childhood seen at our centre compared with 3% in the United Kingdom (Lawson & Steward 1975). The absence of sexual bias supports an autosomal mode of transmission. The mean age of our patients higher than that of children reviewed by Bedford et al (1971) and Kock & Haeser (1979) is probably related to late presentation with more advanced disease and the apparent absence of hereditary cases in our series. Francois et al (1964) have found that non-hereditary cases have a higher mean age at diagnosis than hereditary cases. A family history of retinoblastoma appears to be rare in Swedish reports (Jereb et al 1967, Nordal et al 1973) and supports the suggestion that most cases of retinoblastoma are sporadic (Duke Elder 1967). The absence of a family history in our series is probably because our earlier cases have invariably succumbed to the disease without

Table II

Clinical Details Treatment and Outcome in 20 cases of Retinoblastoma

Case No	Year	Age at Diagnosis	Sex	Pathological Findings	Staging of Disease	Treatment			Outcome
						Surgery	Surgery & R T	Surgery + R T + Chemotherapy	
1	1968	7 years	F	ONI	VI	-	+	-	Bone metastases at 13 months - went abroad
2	1968	2 years	F	ONI	VI	-	+	-	A + W 10 years later
3	1969	2 years	F	data not available	?	-	+	-	A + W 12 years later
4	1970	10 months	M	corneal tags (L) (R) scattered creamy patches opaque media	V (bilateral)	0	0	0	Refused treatment
5	1970	13 months	M	tumour involving half the retina	V	+	-	-	V + W 13 months later L to F U
6	1971	8 months	M	data not available	?	0	0	0	Refused treatment
7	1971	18 months	F	tumour in left half the retina	V	-	+	-	A + W 21 months later L to F U
8	1972	27 d	M	extensive extension in L ONI	VI	-	+	-	A + W 1 year later
9	1972	2 d	V	extensive extension in R ONI	VI	-	-	-	Alive 10 months later

I	1971	30	d	I	ON1	V	ON1	VI	-	+	-	e n b t
13	1971	3 years	F	retro & posterior chamber	ON1	VI	+	VI	+	-	-	A + W t (m ad lost to U
14	1971	90 months	M	ON1	ON1	VI	-	VI	+	-	-	A + W 1 years
15	1975	90 months	M	vitreous seeding	ON1	VI	-	VI	-	-	-	Died within 7 weeks of cerebral & spinal metastases
16	1976	4 years	F	ON1	ON1	VI	-	VI	+	-	+	A + W at 2 years 9 months (NS involvement at 5 months responded to MTV and VAC lost to F U
17	1976	22 months	M	ON1	ON1	VI	-	VI	-	-	+	tumour in opposite eye at 7 months sent for R T lost to U
18	1976	7 years	F	ON1	ON1	VI	-	VI	-	-	+	(NS involvement at 1 years No response to MTV VCR ADM and CIA Died 3 weeks later
19	1976	14 months	F	ON1	ON1	VI	-	VI	-	+	-	lost to U at 2 months
20	1977	8 months	F	(Retro) ON1 (lost to U)	ON1	VI	+	VI	+	-	-	lost to F U at 1 month

ON1 = optic nerve infiltration V + W = alive and well F U = follow up F = radiotherapy MIN = metastatic
VCK = vitreous CIA = chorioretinal ADM = adenoma

an opportunity to reproduce and transmit the gene to their children. The sublocation of the tumour reveals an even greater preference for the right compared with similar reports from the Scandinavian countries (Kock & Naeser 1979). The reason for this is not clear.

Our experience with the treatment of retinoblastoma has been confined to advanced disease. Eight of our 20 children have survived more than one year and for periods ranging from 2 to 12 years. Our cure rate compares favourably with the 29.36% rate reported by Ellsworth (1977). The addition to chemotherapy does not appear to have improved survival and the appearance of tumour during maintenance chemotherapy in 2 cases suggests that the combination used by us is ineffective. It would be more rational to use methotrexate, BCNU, CCNU or DTM which cross the blood-brain barrier but this would need evaluation. The effective treatment currently available for advanced disease appears to be enucleation followed by orbital radiotherapy. The presence of tumour cells in the vitreous chamber or infiltration of tumour into the optic nerve is not necessarily equated with poor prognosis (Kock & Naeser 1979).

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LEBER'S MILIARY ANEURYSM AND DYSBETALIPOPROTEINAEMIA A Case Report

BY

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A 19-year-old male suffering from Leber's miliary aneurysm was also found to be affected by a dysbetalipoproteinaemia (type IV hyperlipoproteinaemia). The pathophysiologic mechanism of the condition and the possible metabolic cause are discussed. In view of such findings, evaluation of plasma lipids in patients with Leber's miliary aneurysm would seem to be warranted.

Key words: dysbetalipoproteinaemia - fluorescein angiography - Leber's miliary aneurysm

As Leber described the disease of miliary aneurysm (Leber 1912) several others have been recorded and evidence has been forthcoming which conveys a relationship between this retinal condition and certain associated systemic disorders. For reasons which are unexplained, the condition appears in young males and is usually unilateral. Many possible causes for Leber's miliary aneurysm have been proposed but there is no conclusive evidence for any of them. It is the purpose of this presentation to describe a patient with Leber's miliary aneurysm not only as an addition to the literature but also as a report on an associated metabolic defect which does not appear to have been previously mentioned.

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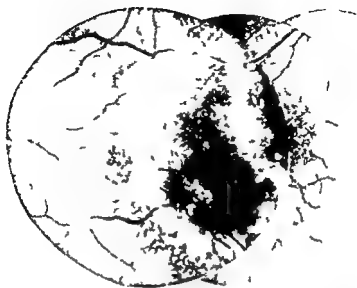


Fig 1

The fundus of the right eye with the bulb-like dilatactions of the inferior temporal artery and the circinate lesion with exudative rings involving the macula.

Case Report

This 19 year-old male was referred to us because of a gradual visual acuity deterioration in his right eye. He had been well until the time that he complained of visual problems which interfered with his studies. He did not complain about any other disease, his past history was essentially negative and a detailed family history showed no evidence of any systemic disease.

When examined vision in the right eye was 20/80 and in the left 20/20. The intraocular pressure and the anterior segment were normal. Abnormal findings were confined to the right fundus. These abnormalities included a patch of fatty exudation deposited in a circinate fashion (Fig 1). These hard exudates surrounded some aneurysmal dilatactions distal to the 2nd and 3rd bifurcation of the inferior temporal artery. These vascular disorders were found only along divisions of the inferotemporal artery, the veins remaining normal in appearance. The walls of the involved arteries were thickened and whitish in the region of these aneurysmal dilatactions. A limiting fibrous layer with mild retinal oedema circumscribed the aneurysms. The dense ring of fatty degeneration crossed the macular region explaining the fall in visual acuity. The retinal arterial tree demonstrated an increased light reflex. The lumen of the inferior temporal artery presented regional narrowing of its calibre. Retinaloscopic examinations revealed the presence of telangiectatic dilatactions of the fine retinal capillary bed adjacent to the circinate area.



Fig 2

keim angiography of the right eye. Left: Early arterio-venous phase demonstrating perfusion of the infero temporal artery and its corresponding capillary bed, highlighting the hypofluorescent patch. Middle: Arterio-venous phase showing telangiectations of the precapillary arterioles and capillary bed. Right: Late phase showing vascular leakage of dye, evidenced by the development of staining surrounding the areas of aneurysms and telangiectatic vessels.

al field demonstrated a scotoma in the right eye without break through into the ery and corresponding to the diseased area. Colour testing gave a defect in the blue axis for the right eye but normal response in the left. Dark adaptation for cone and thresholds slightly elevated in the right eye, was normal in the left. The perimetry presented subnormal amplitudes in the right eye. However, both the static and scotopic components were present.

Fluorescein angiography demonstrated arteries with segmental narrowing and saccular aneurysms along the inferior temporal branches. In the early arterial phase, fluorescein was delayed by these aneurysmal lesions, their diameters being twice that of the involved vessels. The transit of the dye was normal to the site of the aneurysm but presented marked delay in the filling of the artery distally to it (Fig 2 left). Furthermore, the aneurysm acquired a dense fluorescence and a zone of intraretinal leak was found in the area immediately surrounding this. The capillary network was visible and dilated. The branches of the capillary network were visible and dilated. The appearance of multiple hyperfluorescent spots with diameters corresponding to the telangiectatic lesions noted on ophthalmoscopy confirmed the presence of vessels studded with microaneurysms (Fig 2 middle). In the late venous phase of the angiogram (Fig 2 right), a diffuse and dense staining was seen, indicating extensive dye leakage.

leakage from the capillaries and microaneurysms. The vessel wall stained in such a point of the aneurysms retaining the dye longer than the underlying vessels. The area of dye leakage was much more widespread than could be appreciated from the ophthalmoscopic changes.

Since many different causes can initiate the clinical picture of intraretinal and subretinal exudation, detailed investigations were performed in an attempt to correlate eye-laboratory changes with this retinal condition. The standard laboratory tests including plasma cholesterol yielded normal findings. However, the patient's triglycerides were elevated to 250 mg/dl. As such a plasma lipid disturbance was found, further studies with lipoprotein electrophoresis were carried out to fully define the abnormality. A dense pre-beta band in lipoprotein electrophoresis was obtained and on the basis of the elevated plasma triglycerides with normal plasma cholesterol the diagnosis of type IV hyperlipoproteinemia was established. As this type of lipoprotein abnormality with hypertriglyceridaemia is known to be carbohydrate induced, a low carbohydrate diet was initiated, since there is little else to be done. Photocoagulation treatment of the retinal lesions was in the meantime withheld.

Discussion

The pattern of the retinal anomaly in our patient was specific enough to suggest Leber's military aneurysm.

The pathophysiological mechanism underlying this anomaly is not clear, although there are some suggestive observations in patients who have had associated involvements of other parts of the body. The occurrence of such combined lesions would appear to establish the point of view that the disease process in our patient is ocular.

The frequency of unilateral involvement and the absence of any hereditary element lend credence to the belief that Leber's military aneurysm is due to a developmental anomaly occurring as an isolated aberration of retinal vasculature (Wegener 1969). However, simultaneous instances of telangiectasis in the retina and elsewhere in the vascular system were supported by the occurrence of aneurysmal lesions in the retina and capillary disorders in the nasal mucous membrane (Gall & Draf 1975). Evidence for such a nature of the causative lesion was also suggested by a patient with Leber's military aneurysm in the left eye and optic atrophy in the right eye (Archer & Krill 1971). In this case, assumptions were brought forth that a common cause of both conditions with telangiectatic peripheral retinal vessels in one eye and retrobulbar telangiectasis of the nerve head in the other. Several patients have been described with involvements of other parts of the body in relationships to systemic disorders such as epilepsy (Bryson & Wolter 1964), phakomatosis (Paufigue et al. 1964) or Osler's disease (Heifricer & Schwartz 1964).

In other cases the clinical features associated with Leber's miliary aneurysm were such that the possibility of a metabolic factor could be born in mind. In our case a metabolic defect of dysbetalipoproteinaemia type was also found. Assuming Leber's miliary aneurysm is the result of such a metabolic disturbance it is conceivable that xeroglycidaemia per se is not the only etiologic factor. Hyperproteinaemia (Kahan et al. 1964), globulins or particular globulins fractions (Kahan et al. 1964), xogamaglobulinaemia (Frenkel & Russe 1967) and hyperlipaemia with hypercholesterolaemia (Kahan et al. 1964) were thought to be equally important in the aetiology of this disease. Our patient represents an example of a case with normocholesterolaemia associated with abnormal lipoprotein structure but without excess circulating cholesterol. The primary defect appeared to be the occurrence of abnormal beta lipoproteins which contained an excess of triglycerides. Considering the finding from the wider perspective of plasma lipoprotein disturbance rather than the more limited consideration of xeroglycidaemia, it is apparent that such metabolic dysfunctions may be added to the aetiology of Leber's miliary aneurysm.

The pathophysiologic mechanism by which the retinal changes are produced has remained elusive. It has been thought (Houston & Wise 1957) after consideration of their characteristic pattern in circinate retinopathy that these exudates were due to multiple zones of retinal hypoxia secondary to local vascular obstruction. Whether this fatty material arises from dilated capillaries adjacent to hypoxic retinal areas or whether it represents a by-product of the altered metabolism which have leaked from the capillaries and cannot be resorbed is not known.

The fluorescein angiography was helpful in detecting and defining the structural changes in the permeability changes in the affected vessels and in demonstrating the extent of the extravascular leakage. The angiography demonstrated not only the central artery involvement but also the fine capillary bed alteration. The central retinal artery bed showed diffuse as well as focal dilatactions which were manifest on both fundal and bithalmscopically.

It seems that the aetio-pathogenesis of Leber's miliary aneurysm cannot be deduced from present information nor is it clear whether any correlation between its appearance and any systemic disease exists apart from speculation that they may both be due to the same basic abnormality. The possibility that the xeroglycidaemia in our patient may be fortuitous rather than causative or that both the retinal disease and the dysbetalipoproteinaemia may be a reflection of a causally related metabolic defect cannot be excluded.

The true physiological significance of such a metabolic defect remains to be determined and warrants further investigation. Additional cases are needed to confirm and possibly extend the above observation.

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CASE OF MESENCHYMAL CHONDROSARCOMA OF THE ORBIT

BY

MASASHI SHIMO OKU¹ NOBUKO OKAMOTO¹ YOJI OGITA¹

and TERUMASA SASHIKATA²

A case of mesenchymal chondrosarcoma occurring in the right orbit of a 84-year-old Japanese female was reported. The ultrastructural findings of tumour are composed of three cell types: well-differentiated cartilaginous cells, undifferentiated cells and transitional cells. The well-differentiated cells showed scalloped cytoplasmic membranes, numerous mitochondria showing dense electron matrix, various size of lipid granules and abundant amount of extracellular matrix. The extracellular matrix were collagen fibres and ground substances and matrix vesicle. The undifferentiated cells showed smooth cytoplasmic membranes, large nuclei resembling primitive mesenchymal cells.

Key words: 84 year-old patient - orbital mesenchymal chondrosarcoma - undifferentiated - transitional - cartilaginous

Mesenchymal chondrosarcoma is a rare tumour which has been described originally by Lichtenstein & Bernstein (1956). It is generally accepted that 70% of the cases occur in bone and the remaining 30% have originated from soft tissues. To our knowledge, only 12 orbital mesenchymal chondrosarcoma (Dabrowska 1972, Ueda et al 1973, Mizuno et al 1978, Okuda et al 1976, Ramirez et al 1971).

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Reeh 1966 Salvador et al 1971 Sevel 1974 Silva et al 1977 Zielinska & Co 1977) have been reported. Until recently, however, electron microscopic examination of the tumour was not described in all of the reported cases except report. In the present paper the author describes the light and electron microscopic findings in a case of a 84 year-old Japanese female with orbital mesenchymal chondrosarcoma.

Case Report

A 84 year-old woman was admitted to Iizyogo College of Medicine Hospital on 6/19/77 complaining of a 3 year history of gradual proptosis with swelling of the right eye (Fig 1).

A firm smooth surfaced tumour pushed the right eye laterally and upward (exophthalmometer: right 20 mm, left 14 mm) and the eye movement was severely

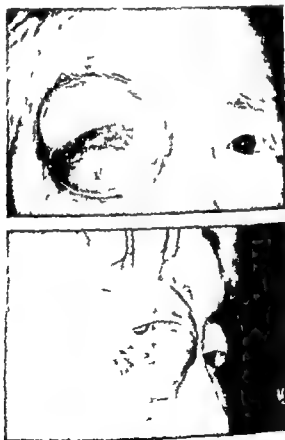


Fig 1
Exophthalmos and eversion of the inferior fornix due to the orbital tumour

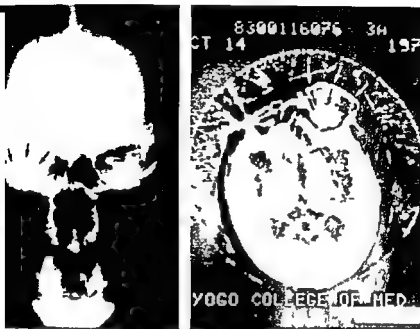


Fig 2

radiodense opacity in the right orbit. High and low density in the right orbit by CT scan

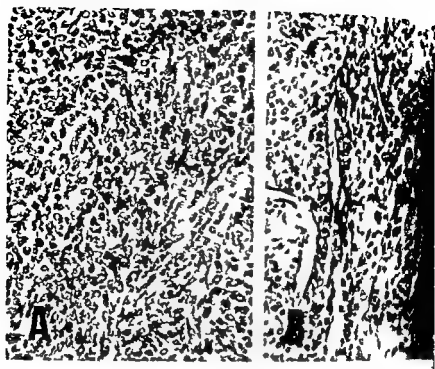
Directions The vision of the right eye was already lost. Roentgenogram examination showed a brain like high radiodense opacity in the right orbit. CT scan also showed two areas of high and low density in the same region (Fig 2). The tumour was extirpated on 19/7/1977.

Gross findings The extirpated tumour was 5x5x3 cm in size and about 42 g in weight. Cut surfaces revealed brownish white elastic hard mass with foci of calcification and grayish white semi translucent nodules.

Methods for histological investigation

Tissue for light microscopy was fixed in 10% formalin, processed, sectioned as usual method and stained with haematoxylin and eosin and other stainings such as alcian blue and silver impregnation.

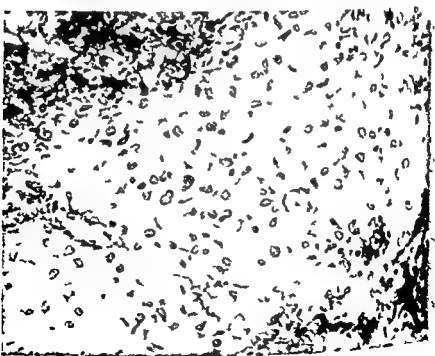
For electron microscopic observations, approximately 1 mm thick blocks were fixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), dehydrated in alcohol and embedded in Epon 812. Ultrathin sections were stained with toluidine blue and ultrathin sections were doubly stained with uranyl acetate and lead citrate and were examined with Akashi S-500 electron microscope.



FR 3 4 B

A Undifferentiated cellular area H.E. $\times 140$

B Vascular pattern resembling haemangiopericytoma, H.E. $\times 140$



FR 4

Higher magnification of a cartilaginous island Alcian blue staining $\times 400$

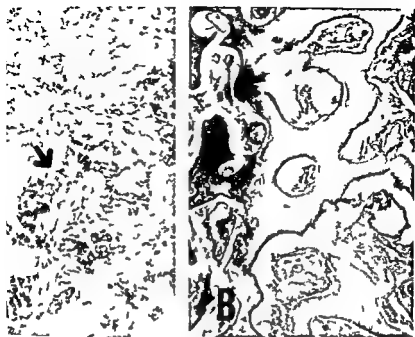


Fig 5 A B

- A Calcification in the center of the cartilaginous island H E \times 140
 B Mature bone formation in the peripheral part of the tumor H E \times 140

Top of findings

The tumour showed two types of histology. Most of the tumour was composed of small round or short spindle shaped cells with scanty cytoplasm and centrally located large nuclei. These tumour cells were often found to arrange around the abundant capillary vessels, resembling the pattern seen in haemangiopericytoma (Fig 3). Among these cellular areas, there were cartilaginous islands of various size with somewhat atypical chondrocytes showing moderate nuclear atypism but rare mitosis (Fig 4). In certain areas, calcification was found in the center of the cartilaginous islands. At the peripheral part of the tumour adjacent to the orbit, mature bone formation was also found (Fig 5).

Structural findings

The undifferentiated cellular area consisted of round or oval cells having large round or oval nuclei (Fig 6). The plasma membrane was smooth. The mitochondria were round in shape. Cytoplasmic organelles such as rough endoplasmic reticulum and free ribosomes were not present in these undifferentiated cells. Most of the round or oval cells were arranged close to each other and the inter-cellular matrix was sparse. The cells representing transitional or intermediate forms between undifferentiated cells and chondrocytes were observed. These cells showed elongated or stellate configuration with

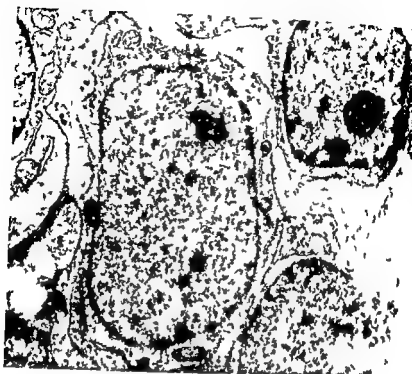


Fig 6

Undifferentiated cells showing scanty cytoplasm with a few mitochondria and endoplasmic reticulum $\times 24100$ bar = 1μ .

several coarse cytoplasmic processes (Fig 7). They were separated from each other by collagen fibers. The characteristic findings of the cytoplasm were as follows: the mitochondrial matrix was higher than that of undifferentiated cells. Furthermore, cytoplasmic vacuoles of various sizes were observed.

Within the islands of well differentiated tumour cells, typical features of chondrocytes were seen (Fig 8). The cell membrane was characterized by a scalloped margin. The nucleus was ovoid with diffuse chromatin. The cytoplasm contained lipid vacuoles of varying sizes, a moderate number of mitochondria showing an electron dense matrix and rough endoplasmic reticulum. Small particles which may be a staining product of lead compound were observed adjacent to the cell membrane.

Discussion

The histological appearance of this tumour was considered distinctive. It consisted of proliferation of primitive undifferentiated mesenchymal cells in which small islands of well differentiated cartilage were present. We found mature

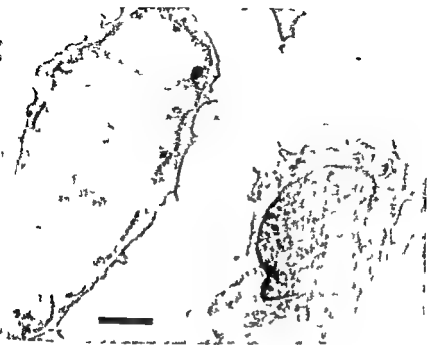


Fig 7

representing transitional form with several coarse cytoplasmic mitochondria with denser matrix $\times 17,000$ bar

formation in the peripheral area of the tumour Salvador et al (1977) and our findings. We assumed that this bone formation was a result of the well-differentiated cartilaginous islands because of the long duration of the tumour.

Mesenchymal chondrosarcoma closely resembles haemangiopericytoma, rhabdomyosarcoma, fibrosarcoma and ordinary chondrosarcoma and it is sometimes difficult to differentiate from them. When the cartilaginous area is observed it is difficult to differentiate from haemangiopericytoma and from reticulum cell sarcoma. The cells of fibrosarcoma are larger than those of mesenchymal chondrosarcoma. The cartilaginous cellular elements of ordinary chondrosarcoma are larger and more pleomorphic than those of mesenchymal chondrosarcoma.

Ultrastructural studies of the tumour were reported by several authors (Fu & Kay 1977; Mikata et al 1977; Mizuno et al 1978; Stemer et al 1973). Mizuno et al (1978) have reported ultrastructural aspects of orbital mesenchymal chondrosarcoma, however the description was limited to the undifferentiated cellular area. In the present case electron microscopic features have shown three types of tumour



Fig 8

Well differentiated chondrocytic form of tumour cell showing scalloping processes probably vacuoles (arrows) and pericellular matrix $\times 93\ 000$ bar = 1μ .

cells undifferentiated cells transitional cells and cartilaginous cells Fig 9 shows scheme of relationship between these tumour cells. As for the undifferentiated cells the present observations are in agreement with those of previous reports. However as for the transitional or cartilaginous type of tumour cells no different features were shown compared with those of Steiner (1973) or Maki (1977) report. Namely in the present case mitochondria showing electron dense matrix and various sizes of lipid droplets were found in almost all transitional cartilaginous cells.

According to Hay (1978) and Jakobić & Tannenbaum (1974) orbital mesenchyma are classified in four groups primary secondary haemopoietic and neural mesenchyma and secondary mesenchyma are derived from both mesoderm and neural crest. Tumours originating from the secondary mesenchyma are fibrosarcoma osteosarcoma chondrosarcoma leiomyoma sarcoma haemangiopericytoma rhabdomyosarcoma and liposarcoma. Therefore the origin of this case seems to be secondary mesenchyma though not definitely from mesoderm or from neural crest.

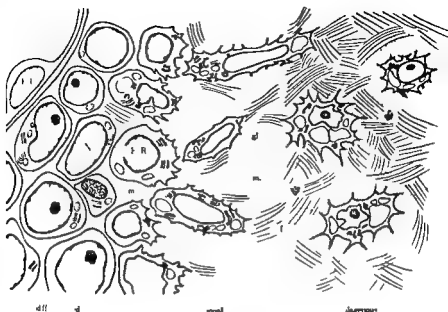


Fig 9

hema of the mesenchymal chondrosarcoma Cap Capillary End Endothelial cell N nucleus r E R rough Endoplasmic Reticulum mit mitochondria glv glycogen m matrix vesicles c f collagen fiber

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UNUSUAL COURSE OF PAINFUL OPHTHALMOPLÉGIA REPORT OF A CASE

BY

BO I ANDERSSON

A case of Tolosa Hunt syndrome with unusual course is described. Pain and ophthalmoplegia were both relatively late manifestations preceded for several weeks by progressive involvement of the optic nerve. The importance of also bearing this condition in mind in atypical cases is stressed, since early diagnosis and corticosteroid treatment distinctly reduce the risk of severe residual symptoms.

Key words: Tolosa Hunt syndrome — painful ophthalmoplegia — superior orbital fissure syndrome — oculomotor paralysis — cavernous sinus syndrome — orbital apex syndrome

The symptom complex of painful ophthalmoplegia may be attributed to various causes amongst which the most important are carotid aneurysm, collagen disease, diabetes, neoplasms, specific granulomatous conditions and so far unspecified inflammatory processes. The Tolosa Hunt syndrome exemplifies an inflammatory condition which is not well defined pathologically owing to the fact that only a few occasional cases have been histologically examined (Tolosa 1954, Lakke 1962, Schwartz & Farmer 1972, Levy et al. 1975). Nevertheless the disorder shows striking and often uniform clinical features representing a clinical entity (Smith & Taxdal 1976). The prompt effect by corticosteroids upon in particular the pain has been

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emphasized (Smith & Taxdal 1966 Mathew & Chandy 1970 Hunt 1976). This effect however is non specific and can only be used as a diagnostic criterion after thorough investigation (Fowler et al 1975).

All neurological structures in the region of the cavernous sinus, the superior orbital fissure and the orbital apex may become involved to various extent and in any succession in the Tolosa Hunt syndrome. However in the vast majority of reported cases pain and ophthalmoplegia were early symptoms and appeared in close connection with each other. Notter's (1977) review disclosed only 5 out of 33 cases where pain was a late symptom or did not occur at all. The optic nerve is rarely involved.

The aim of the present case report is to call attention to this diagnosis even in cases where both pain and ophthalmoplegia are late manifestations.

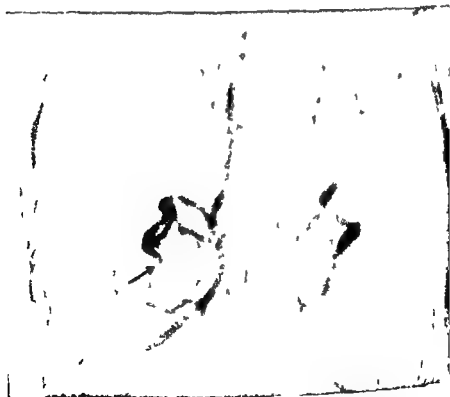


Fig 1

Orbital phlebography July 3 antero-posterior view. No filling of the posterior part of right superior ophthalmic vein (arrow) and scant filling of the cavernous sinus on the same side.

FPA
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Fig 2

Fig 2. Lateral view angiography August 1. Slight local narrowing of the proximal intradural part of the carotid siphon (arrow)

Case Report

This is a 52 year-old man previously healthy and with no history of systemic or ocular disorder. In 1976 he had a 3 week episode of pulsating pain around his right eye with no objective neurological dysfunction. In the middle of April 1978 the patient noticed a decrease in objective colour vision. The right central visual field was blurred and vision was further impaired with further progress during the following month. In the beginning of May 1978 he was examined by an ophthalmologist who found marked reduction of visual acuity and a large central scotoma in the right visual field. In the right fundus the optic disc was swollen with peripapillary bleedings and slight segmental venous distension. The peripheral retinae were normal. IOP was bilaterally normal. The ESR was below 10 mm/h. The condition was interpreted as a central venous thrombosis. From the middle of May 1978 there was progressive right sided retro-orbital pain, intense and piercing in character. Later ptosis developed and oculomotor dysfunction due to involvement of the third, fourth and sixth nerves was noticed. The ophthalmoplegia progressed as did the visual impairment, and at the end of June the right eye was amaurotic and there were total sixth nerve palsies. Pain was unbearable and on ophthalmological examination papilloedema of 6 to 7 dioptres, ptosis and slight proptosis furthermore were

observed on the right side. Treatment was given with 30 mg of prednisone daily. Pain vanished within a couple of days and there was slight but temporary improvement of vision and oculomotor function. An intraorbital expansive process was suspected, and orbital phlebography on July 3 disclosed obliteration of the posterior part of the right superior ophthalmic vein and of the right cavernous sinus (Fig 1). Roentgenographic examinations of the skull and face were normal including orbital tomography. A right carotid angiography on August 1 showed slight local narrowing of the proximal intradural part of the carotid siphon (Fig 2). An electroencephalogram was normal. Inspection of the epipharynx was negative as were serology for lues and immunological screening including antinuclear factor. On August 3 the ESR was 44 mm/h. The steroid treatment was discontinued in the beginning of September and only a few days later pain recurred.

On October 23 the patient was admitted to the neurological clinic. Examination revealed total amaurosis and complete third and sixth nerve palsies on the right side. Vision was intact. The fourth and fifth nerves were normal. There was atrophy of the right optic disc and the right pupil now was of intermediate size reacting neither directly nor consensually to light. The ESR was 38 mm/h but routine laboratory examinations otherwise were normal including



Fig 3

Right carotid angiography October 31 lateral view. The local carotid narrowing is slightly more accentuated.

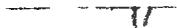


Fig 4

nal phlebography November III antero-posterior view. Somewhat better filling of the posterior orbital collaterals on the right side, otherwise unchanged.

and glucose. On suspicion of Tolosa Hunt syndrome corticosteroid treatment was again started with 30 mg of prednisone daily. Five hours later the patient was free of pain. The following month oculomotor function was partly restored and ptosis was completely resolved, but amaurosis remained. Angiographies were repeated and right carotid angiography on October 31 showed some progress of the local narrowing of the carotid siphon (Fig 3) whereas orbital phlebography on November 6 principally was unaltered (Fig 4). Local course and ESR development are illustrated in Fig 5.

In November III the patient developed massive bilateral pulmonary embolism, the source being a clinically silent deep venous thrombosis of the right leg. Treatment was given with heparin and warfarin sodium, but the latter was discontinued because of a gastric haemorrhage. The question was arisen whether these complications were expressions of a generalized leucus-vasculitis. However, this was not verified and a right temporal artery biopsy was made (undertaken during steroid treatment) as was coagulation status (antithrombin III). Attempts to withdraw steroid treatment resulted in a rise of ESR indicating a persisting activity of the inflammatory retro-orbital process. The patient was discharged on December

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Short Communication

**SNAKE LIKE APPEARANCE OF NUCLEAR CHROMATIN
IN CONJUNCTIVAL EPITHELIAL CELLS FROM PATIENTS
WITH KERATOCONJUNCTIVITIS SICCA**

BY

KIRSTEN MARNER

Millipore filter mprints of conjunctiva in patients with conjunctival symptoms revealed a snake like appearance of the chromatin in nuclei of epithelial cells. A total of 40 patients were investigated. Abnormal cells were found in 8/16 patients with long term keratoconjunctivitis sicca and in 6/24 patients suspected for Sjögrens syndrome. The abnormal cells were typically arranged in clusters among morphologically normal epithelial cells. The finding was significantly correlated to the severity of the disease in patients with keratoconjunctivitis sicca. In all patients the abnormal cells could only be detected in millipore mprints of the upper bulbar conjunctiva and not in scrapings from the same area. The significance of the findings are discussed.

Keywords: keratoconjunctivitis sicca, Sjögrens syndrome, nuclear chromatin, conjunctival epithelial cells.

The use of millipore filters has recently been introduced in ophthalmology as a surface biopsy (Egbert et al 1977). The method is claimed to be simple, gentle and reported to give a representative harvest of cells.

Patients with keratoconjunctivitis sicca have dry and tender eyes and a conjunc-

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ual biopsy is therefore painful and may be complicated. As the millipore imprint technique was found to be easy it was applied on the conjunctiva of keratoconjunctivitis sicca patients.

Material and Methods

Thirty five normal persons (70 eyes) were used as controls. Sixteen patients (32 eyes) with various degrees of keratoconjunctivitis sicca were examined 4-12 times monthly intervals. Twenty four patients (48 eyes) with collagen disease were examined once for the diagnosis of Sjogren's syndrome. All patients and controls were investigated with Schirmer's test (basal secretion) (Norm 1966), break time (BUT) (Norm 1974), rose bengal staining (Norm 1977) and the millipore imprint technique. Conjunctival scrapings were performed with a platinum spatula and stained as described for millipore filters.

Millipore filters (MF) type VS with pore size of $0.02\text{ }\mu\text{m}$ were used. In order to remove water soluble surfactants from the pores of the filters they were soaked in cold water for 30 min and air dried. The filters were cut into a 3 mm round piece and placed on a plastic (PVC) rod by use of double sided adhesive tape. The mounted filter was pressed against the bulbar conjunctiva with a pressure of 25 mmHg for two seconds, removed and fixed by Pro-Fixx® (Aerosol Chemical Fixative) or only air dried. To achieve the constant pressure the plastic rod with filter was mounted on a tensiometer (Correx, Haag Street A.G.). The procedure was performed with and without topical anaesthesia (oxubuprocaine Novem® 0.1%) with the same results. When dry the filter was stained with PAS (periodic acid-Schiff) and Mayer's hematoxylin. After staining the filters were placed on a slide at room temperature and air dried (one hour), cleared with xylol and covered before light microscopy. In six cases the changes found in the chromatin pattern were further investigated by Feulgen's nuclear test.

Statistical analysis of the results was carried out using the Mann-Whitney U-sum test.

Results

During investigations performed to evaluate morphological differences of epithelial cells in various areas of the eye, nuclear changes were surprisingly found in some cells by use of the millipore filters. However, these changes possibly induced by millipore filters showed certain localisation characteristics and restricted to certain patients.

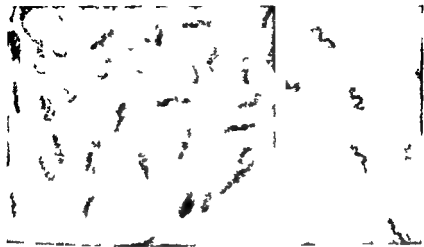


Fig 1

veral stages from normal cells to straight line and snake like appearance of the chromatin in nuclei of the epithelial cells of the conjunctiva

Fig 2

characteristical snake like appearance of epithelial chromatin from a patient with severe keratoconjunctivitis sicca.

ch figures are stained with periodic acid Schiff and hematoxylin (Magnification 1200 \times)

The nuclear changes in epithelial cells are illustrated in Figs 1 and 2. The nuclear chromatin seemed to be condensed in a metaphase like pattern although the nuclear membrane was clearly visible. Furthermore the picture was fairly homogeneous showing no prometaphases or anaphases. However various intermediates are seen between a straight axial and a "snake like" appearance in different patients. In one patient there was a predominance of one type. By use of Feulgen's staining (Feulgen's nucleal test) it was secured that all DNA was contained in the described configurations.

These abnormal epithelial cells were characteristically clustered and the clusters consisted of a few cells to more than 50% of all cells. The unprints containing these normal cells were always from the upper bulbar conjunctiva. Until now the abnormal cells have not been found in light exposed conjunctiva, the lower bulbar conjunctiva or the palpebral conjunctiva.

In 16 patients with keratoconjunctivitis sicca this phenomenon could repeatedly be demonstrated in eight patients in 1/3-1/2 of all epithelial cells at every magnification. Furthermore the abnormal cells were characterized by the snake

like type and were found in both eyes. The abnormal findings in this group of patients were significantly correlated to the severity of the sicca syndrome (Fig 3).

Patients with abnormal cells were investigated with scrapings from the upper bulbar conjunctiva besides the millipore imprint technique and in no cases could the abnormal cells be found by this method.

Twenty four patients were investigated once under the suspicion of Sjögren syndrome. 15/24 of these patients had dry eye symptoms and pathological results of Schurmer's test, BUT and rose bengal staining. Of these 15 patients with dry eye symptoms, six had abnormal cells with typical snake like appearance. In the patients there was no significant difference in the severity of disease in relation to the findings of snake like chromatin in the epithelial cells.

Preliminary findings in other patients indicate that the abnormal cells are restricted to keratoconjunctivitis sicca patients. Thus for instance abnormal cells were found in a number of patients with various forms of allergic conjunctivitis.

With one exception no abnormal cells were found in the group of non-controls. The one exception was a pregnant female with predominance of straight line type in a few cells.

No correlation was found to the use of various artificial tears or to treatment with parenteral steroid.

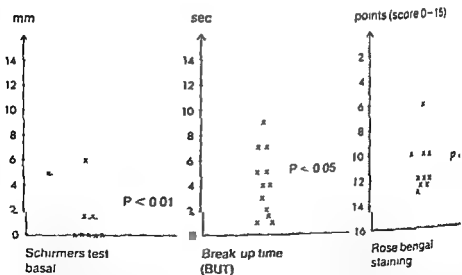


Fig 3

The results of Schurmer's test (basal), break up time and rose bengal staining in keratoconjunctivitis sicca patients (32 eyes). The symbols x or ■ represent mean values of 4-7 months investigations and indicate findings of respectively snake-like chromatin or normal chromatin pattern in epithelial cells. Mean values of the two groups of eyes are indicated by a dotted line and the P values between these values shown.

Discussion

The observed pattern of nuclear chromatin seems undescribed in cellular pathology. It is possibly a special nuclear change during cell death caused by some toxic factor. The chromatin arrangement is not an expression of metaphase since the nuclear membranes are intact and no prometaphases or anaphases were observed.

The toxic factor could be derived from the millipore filter since the abnormal cells were never observed in scrapings. However, millipore filters are used for the culturing of other cell types without seeing the described changes. Furthermore, we had to expect that a possible toxic factor from the millipore filter would equally affect all epithelial cells and if so the described correlation between the severity of keratoconjunctivitis sicca and the limitation to the upper bulbar conjunctiva would be inexplicable.

The findings of abnormal cells were significantly correlated with the severity of disease. However, no correlation was found to the presence of antibodies and factors, particularly ANA. The observed changes might be a result of a direct relationship between the drying procedure of the diseased and healthy cells. The electron micrographs of the present study and the number of snake like nuclei might be used as a prognostic marker. However, the significance of the described findings are under further investigation.

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TRANSACTIONS OF THE SWEDISH OPHTHALMOLOGICAL SOCIETY 1979

EDITED BY

ULF STENEVI

Meeting in Skovde September 14-15 1979

S Stenkula & G Tornquist *Intrectomy in retinal detachment*

T Jerndal & M Lundstrom *300 trabeculectomies a follow-up study through 10 years*
Trabeculectomy holds its place as an efficient and safe operation in open angle glaucoma. In this study postoperative medical therapy was needed in an increasing extent with time.

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Jerndal T & Lundstrom M (1977) 330 trabeculectomies a follow up study of 10 years. *Acta ophthalmol (Kbh)* 55: 52

Jerndal T & Lundstrom M (1980) *Acta ophthalmol (Kbh)* 58: in press

B Lundh & G Lennerstrand *Grating tests in the screening for early glaucoma*

We have determined contrast sensitivity to sinusoidal gratings in patients with glaucoma in order to see if we could confirm the findings of Arden & Jakobsson (1978) that patients with early glaucoma and ocular hypertension often showed a subnormal contrast sensitivity and that the degree of abnormality varied with the grading of the disease.

Methods We tested one group of patients with manifest glaucoma and one group of normals in the same age range. All glaucoma eyes had normal visual acuity (Snellen acuity 0.9-1.0). We determined contrast threshold for sinusoidal gratings of different spatial frequencies with the Arden plates and with the pattern displayed on the screen of a cathode ray tube (CRT). We have previously used the CRT method quite extensively in a clinical setting and found it to be of high reproducibility.

Results With the Arden grating test we found that two out of eleven eyes selected randomly from our eleven normals scored above the normal limit given by Arden. Three normals showed a difference between the two eyes that exceeded what Arden considered as normal. In patients with bilateral glaucoma the most affected eye was selected for this study. Five out of fifteen glaucomatous eyes were found to have normal contrast sensitivity to Arden gratings. Eleven of the eyes had visual field defects and four of these scored as normals. With the CRT method we found normal contrast sensitivity in eight out of ten eyes with pathological Arden score. This group included eyes with visual field defects. One eye with visual field defects had a subnormal CRT contrast sensitivity but a normal Arden score.

Conclusion Since we were unable to safely differentiate normal from glaucomatous eyes with either of the contrast sensitivity methods we conclude that determination of grating thresholds cannot be used as a reliable screening test for early glaucoma.

Reference

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Naeser Benign mixed tumour of the lacrimal gland in a 10 year old boy: a clinicopathological report

A slowly growing tumour underneath the lateral part of the right upper eye lid was observed for about three months in a 10 year-old boy. The tumour was not adherent to the surrounding tissues. Fine needle biopsy displayed monomorphous cells which in many places were surrounded by eosinophilic fibrillar mucus. There was no cellular atypia. The histological diagnosis was benign mixed tumour of the lacrimal gland.

Computerized tomography showed a highly attenuating process in the anterior and lateral part of the right orbit between the eye globe and the lateral orbital wall. There was no retrobulbar extension of the tumour.

At operation the tumour was removed through the upper eye lid. It had a capsule and was fixed to the lateral part of the lacrimal gland. The tumour had a firm consistency and measured 15 x 14 x 14 mm. The cut surface was grayish white. The histopathological slides showed the typical picture of a benign mixed tumour with epithelial cells in cords, ducts and acinar like formations. In the loose stroma there were myxomatous and chondromatous areas. Spino-cellular metaplastic changes were occasionally seen. There were however no cellular atypia and the whole tumour was surrounded by an intact connective tissue capsule.

The present case shows that benign mixed tumours may be found at the age of ten. The case also illustrates the great help of fine needle biopsy for the diagnosis of orbital tumours.

Symposium

Beta Blockers in the Treatment of Glaucoma

Panel members B Calusendorff, M Pandolf, C Tinnquist, A Wetter, H A Öhrström

P E Wälinder, F B Håkansson & M Lindberg Timolol ophthalmic solution in the treatment of exfoliative and simple glaucoma.

The hypotensive effect of Timolol ophthalmic solution was studied in previously untreated open angle glaucoma (preliminary results were presented at the Swedish ophthalmology meeting 78 09 29).

The study included 68 eyes in 30 patients mean age 68 years. There were 30 eyes with glaucoma and 18 simple glaucoma with an average intraocular pressure (IOP) before treatment of 30 and 28 mmHg respectively. Most eyes had visual field defects and glaucomatous changes of the optic disc.

Timolol caused a marked lowering of IOP initially in exfoliative glaucoma, especially in eyes with high IOP before treatment. The hypotensive effect then decreased and only 5 of 48 eyes (33%) were controlled (< 22 mmHg) by Timolol alone during nine months, 17 (40%) required additional therapy and 12 eyes were operated on. One eye was controlled with pilocarpine alone.

Fourteen simple glaucoma eyes were followed for nine months. In eleven eyes (93%) was controlled with Timolol alone, one eye required additional pilocarpine and two eyes were operated on.

In 15 eyes the treatment with Timolol was stopped. IOP measurements one to five years later showed an average increase of IOP of 5 mmHg.

Three eyes showed a slight punctate keratitis during the treatment with Timolol, the drug was discontinued in one eye. One 65 year-old patient developed a cystic macular oedema, one eye during treatment with Timolol, the drug was discontinued.

Conclusions In our material Timolol alone controlled IOP in most simple glaucoma. In exfoliative glaucoma often required additional therapy.

Meeting in Stockholm December 5-7 1979

Symposium

Clinical applications of recent advances in visual physiology

Moderator G Lennerstrand

III Granit *Information processing in the visual cortex: basic aspects*

G Lennerstrand *VEP and visual function*

Clinical testing of visual evoked potentials or VEP is usually done with pattern reversal stimulation of grating or checker board shape. An increase in latency of transient VEP is a diagnostic sign for optic neuritis (Halliday et al 1973). Steady state VEPs have been used for refraction (Millodot & Ruggs 1970), detection of visual field defects (Cappin & Young 1971) for electrophysiological tests on meridional amblyopia (Fiorenini & Maffei 1971) and strabismic amblyopia (Sokol 1977).

VEP can also be used for testing binocular vision. A method based on binocular rivalry has been developed in which prominent VEP depression in dioptic stimulation is a sign of normal binocular functions and an unchanged binocular VEP in comparison with the monocular response was the mark of disrupted binocular vision due to squint or binocular rivalry (Lennerstrand 1978).

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J Jakobsson A comparison of different VEP methods for assessment of binocular functions

Two VEP methods have been compared, regarding their ability to assess binocular functions. One method is assumed to reflect binocular competition in the striate cortex (as described above by Lennerstrand) and the other neural summation in the visual areas of the signals from the two eyes.

Methods The stimulator is a transparent screen made of squares of polarizing material subtending 30 min of arc at the viewing distance. In the first method suggested to reflect binocular competition the pattern was viewed through two polarizing discs, one for each eye. The discs rotated at different speeds inducing pattern reversal at slightly different rates for each eye. The responses were fed through two amplifiers, each phase locked to one stimulus rate. In this manner it was possible to record responses from each eye even during binocular stimulation. With the second method, based on binocular summation, the pattern was viewed through one and the same polarizing disc for both eyes. The binocular response in this method represents the combined VEP of the two eyes.

Results Eighteen subjects were tested with both methods. Ten had normal binocular vision and eight defective binocularity. The binocular/monocular ratio was calculated. This ratio was compared with the subjects' stereoscopic acuity measured psychophysically. The first method showed fairly good correlation with stereoscopic acuity (corr. coeff. + 0.74) while the second method showed poor correlation (corr. coeff. + 0.34). A negative correlation was expected in the second method.

Conclusion A VEP method suggested to evaluate the state of binocular competition in the striate cortex seemed to be the best for assessing the degree of binocular vision and for separating the individuals with normal binocularity from the ones with defective binocular vision. A VEP method thought to reflect neural summation in the striate cortex seemed less useful in this respect.

J Sjostrand Contrast sensitivity determinations in visual disorders

Determination of the contrast sensitivity function supplements the traditional acuity measurements in quantifying the visual loss for objects larger than the resolution limit. In general, two types of CSF impairments have been demonstrated, one comprising only the

Results The mean fluorescein concentration in the middle of the vitreous body one hour after fluorescein injection in the 11 nondiabetic controls was 4.4 ± 3.7 ng/ml. A fluorescein concentration greater than the mean + 2 SD of controls was regarded as abnormal. An abnormal leakage as found in 15 out of the 26 diabetics. The mean fluorescein concentration one hour following injection in the 26 diabetics was 18.3 ± 18.0 ng/ml. Diabetics who were beyond partial remission had a more pronounced leakage (91.5 ± 18.8 ng/ml) while the diabetics still in partial remission showed no such abnormal leakage (3.5 ± 5.0 ng/ml). There was no significant correlation between a defect blood-retinal barrier and age at onset or duration of the disease. Two patients had an abnormal leakage after a duration of the disease less than 2 years while other patients with a duration of more than 15 years did not have an abnormal barrier. There was no relation between a defect blood-retinal barrier and the actual blood glucose value during the examination. The diabetic with an abnormal blood-retinal barrier had a lower glycosuria index during the last year indicating an inadequate metabolic control in comparison with those patients who had a normal barrier.

In conclusion vitreous fluorophotometry seems to be a very sensitive method for the detection of blood-retinal barrier changes. A strict metabolic control may prevent or postpone the appearance of these early retinal changes.

Symposium Bleeding in Ocular Surgery

Moderator *M Pandolfi*

Panel members *P Algert T B a n s e n S Stenk la S O t t e r l a n*

M Pandolfi Bleeding in ocular surgery

Haemostatic disorders may cause profuse bleeding at ocular surgery. Patients with congenital defects of haemostasis are generally known in Sweden and can be operated upon under protection of substitution treatment. Acquired disorders of haemostasis are more common and often undiagnosed. Of special interest are the defects following treatment with aspirin and other non-steroid anti-inflammatory agents. These substances worsen haemostasis by inhibiting the production of thromboxan which is a potent platelet aggregant. These drugs should be withdrawn one week before operation.

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1979 Year Book of Ophthalmology edited by William F. Hughes Pp 366 with illustr Year Book Medical Publishers inc Chicago-London Price dollars 39.75

The Year Book of Ophthalmology 1979 resembles the previous volumes in appearance and quality, having the same size and solid binding, and being again printed on glazed paper. The abundant collection of articles is organized in 14 chapters comprising Lids, Lacrimal Apparatus and Orbit, Motility, Vision, Refraction and Contact Lenses, Conjunctiva, Cornea and Sclera, Glaucoma, Lens, Lids, Vitreous, Retina, Neuro-ophthalmology, Medical Ophthalmology and Drug Therapy, Surgery and Basic Sciences, and Miscellaneous. A final 15th chapter contains 49 questions concerning the subjects treated of in the previous 14 chapters. By trying to answer these questions the reader has a chance of controlling whether he/she has obtained full benefit of reading the book. If not, the questions may serve for and lead to a recapitulation of some of the read chapters. The contents seem to have been chosen with great care and with a suitable classification within the various groups of subjects.

Reading of the book, therefore, contributes towards prompt and easy information on the latest conquests within the various fields of ophthalmology, including practical therapeutic and surgical problems as well as theoretical problems.

The great majority of the chapters are kept in a concise form. However, many of them have references of aid to those who want to go into further details regarding a special subject. At the back of the book we find a good subject index and an equally good index to authors.

I can accordingly recommend the Year Book of Ophthalmology 1979 to all ophthalmologists and others interested in ophthalmology who in the daily routine feel that they have no possibility of keeping sufficiently up to date with the current ophthalmological literature in journals and recently published books. The book under review gives the reader an easy chance of being informed about the latest achievements within ophthalmology. To those who are particularly interested and engaged in a special ophthalmological problem the book will hardly bring any new details on this subject, but the reader may then enjoy going through the other chapters.

A. E. Rasmussen

Dr. H. E. Dieckhoff M. Dieckhoff: Die Glaukome in der Praxis. 3rd ed. Springer Verlag, Berlin Heidelberg New York 1979. DM 19.80.

The author's intentions with this book, to guide the ophthalmologist in private practice and to inform about practical problems concerning diagnosis and therapy of glaucomas in their different forms, has been fulfilled by this pocket book with 6 tables and 64 figures. Dr. Dieckhoff's second intention, that the book should provide some sort of information guide or many patients, seems not to be convenient in a Danish population not familiar with medical terms.

Any field of glaucoma except the scientific is commented on. The author's style is clear and concise and is mostly based on many years of practice.

This is a useful book for the training as well as for the practicing ophthalmologist.

At a time when no really satisfactory procedure or treatment exists for glaucoma, it seems necessary to have a firmly guiding companion which is found in this book. Through including some of the latest modern techniques such as tomography, the book is sufficiently up-to-date to mention some of the new pharmacological preparations such as timolol and to the new modern forms of drainage operations.

Torben B. Sørensen

Studj A Rollungsschulen physiologie - instrumentarium - therapie Verlag C. W. Kiesweg 23 D-6300 Cressen ISBN 3-902306-00-4 113 pp. 35 pictures Price 26 -

The title of this book *cyclodeviations - physiology instrumentis and therapie* is promising. Certainly there is a need for a monograph covering this complex field of strabismology.

After commenting on the classical views on the mechanism behind ocular rotations, the author describes the mode of investigating cyclodeviations in the well-known clinic. He emphasizes that an investigation using only synoptophore or synoptometer does not give a complete picture of a cyclodeviation. The author believes that the proper treatment should be based on the results of several methods. He has designed three new methods and he also stresses the importance of an analysis of after images in combination with synoptometer measurements. Some pathological cases are reviewed and the most important surgical techniques are discussed.

Those strabismologists who are familiar with the vocabulary of the Cressener Schule find this monograph enjoyable. Those who do not will have difficulties in penetrating it with its many confusing passages where physiology, pathology and short case histories are mixed up. There are many shortcomings and the few statistics are never based on cyclodeviations. Those ophthalmologists who had expected a profound discussion of the role of cyclodeviations - as the text title suggests - will be disappointed. The only recommendation of a multianalysis of cyclodeviations in all types of new acquired oculomotor pares, horizontal phorias, A & V syndroms and many other cases, can be of theoretical value but is definitely not of practical importance.

The short and careless English summary is not a translation of the German one. It is a general introduction to the problems and it gives only a vague conception of how cyclodeviations are handled in Cressen.

Helveton Eugene M & Frest D Ellis. Pediatric Ophthalmology Practice. C.V. Mosby Co. Louis Toronto London 1980. 303 pages incl. subject index and references. 250 comprehensive series of figures (about every second page). Price 43.50 dollars.

The book comprises 14 chapters, each representing a cornerstone of examination, diagnosis and therapy within the various of pediatric ophthalmology. There are chapters dealing with sight tests, strabismus, amblyopia, cataract disorders of the lacrimal system, glaucoma, ptosis, diseases of the orbit and retinal diseases in the later group. The main stress laid on retrolental fibroplasia, retinoblastoma and signs of dystopia. The book also contains sections on infections, developmental defects, genetics, anisometropia, dyslexia.

Of course, the book being of a limited size, does not engage in detailed discussions of numerous different complexes of problems. It crystallizes the authors' great clinical and practical experience in pediatric ophthalmology, being a comparable to an excellent, sensible and extremely well illustrated guide book.

The book will doubtless be used even so much as to become a textbook in pediatric ophthalmology all over the world because with its concise text and comprehensive excellent illustrations it represents modern highlights of pediatric ophthalmology.

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THE VALUE OF THE OSCILLATORY POTENTIAL IN SELECTING JUVENILE DIABETICS AT RISK OF DEVELOPING PROLIFERATIVE RETINOPATHY

BY

SVEND ERIK SIMONSEN

A prospective long term study of the predictive value of the oscillatory potential in the development of proliferative diabetic retinopathy has been made in 137 diabetics. Follow up 6-8 and 13-15 years later demonstrated that recording the oscillatory potential in juvenile diabetics with a disease duration of more than 5 years is valuable in selecting those at risk of developing proliferative retinopathy within 5 years at any rate. The predictive value of the oscillatory potential is probably more limited in women who later become pregnant.

Key words: diabetic retinopathy - electroretinography - oscillatory potential

Investigations made by Francois & De Rouck (1954) Karpe et al (1958) and Straub (1961) have shown that the classical ERG does not exhibit significant changes in diabetic retinopathy until very advanced retinopathy is present.

In 1962 Yonemura et al by means of another electroretinographic technique showed that the so called oscillatory potential, a fast oscillating component of the ERG, was selectively reduced even in very early stages of diabetic retinopathy. These findings have since been confirmed among others by Sugita et al (1963) Simonsen (1967 and 1968) Kojima et al (1966) Jacobson et al (1967) Nakajima et al (1968) Brunette & Desrochers (1970) Tassy et al (1971) Armington (1974) and Osterberg (1974).

In order to evaluate whether the oscillatory potential is of prognostic value in diabetic retinopathy a prospective study was started in 1964.

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Materials and Methods

The initial examination was carried out during the period 1964 to 1966 at the Steno Memorial Hospital Gentofte, Denmark, while the final examination was carried out at the Fysio Department, Gentofte Hospital.

141 patients representing 272 eyes were included in the study. Eighteen patients (8 eyes) were later excluded from the material due to refractive errors or age exceeding the limits stated in the protocol. The corrected material therefore comprises 167 eyes from 137 patients: 57 women and 80 men, with insulin-dependent diabetes mellitus aged 17 to 56 years who were consulted at regular intervals at the Steno Memorial Hospital, a hospital for diabetes. In all cases the diabetes had been diagnosed prior to the age of 40. Only 9 of the patients had a corrected visual acuity $\leq 6/12$, the poorest value 6/36 being found in an eye with proliferative retinopathy. All were treated with insulin and diet. Only few of the patients showed metabolic dysregulation at the time of examination. Patients with refractive errors exceeding three dioptres, eye diseases other than diabetic retinopathy, consumption of drugs known to influence the electroretinogram, and pregnant patients were primarily excluded. The ophthalmological examinations included refraction and corrected visual acuity, slit lamp examination, ophthalmoscopy, colour photographs comprising the central 60° of the fundus and the electroretinography.

Prior to the study the normal range of the oscillatory potential was established by examining 50 eyes from 26 healthy non-diabetics aged 17–50 years with normal visual acuity, refractive errors less than three dioptres, normal ophthalmoscopy and normal color vision measured by Ishihara plates.

Electroretinography was carried out after 20 min of dark adaptation preceded by at least half an hour in the examination room at an illumination of about 5 lux. Prior to recording each patient had the procedure thoroughly explained since perfect relaxation is essential for proper performance of the test. Maximal dilatation of the pupils was achieved using cyclopentolate 1.0% and neo-synephrine 10%. The light stimuli were produced by a set Xenon stroboscope using a flash energy of 0.1 J and 0.6 J. Routine recording of the oscillatory potential was done using a flash energy of 0.6 J. The flash duration was about 100 microsecond, the peak luminance at 0.6 J was about 3×10^6 lux measured at the cornea. Flash intensity was varied in order to elicit maximal amplitudes of the oscillatory potential (Møller 1972). The circular mat glass patch of the reflector is equipped with a small red central fixation light. The stimulating visual angle of 39 degrees. The electrical contact lens electrode from both eyes using the Harpe contact lens electrode. The reference electrode was placed on the middle of the earlobe. The potential was led to an amplifier and recorded by photographing 3–5 superimposed cathode ray oscilloscope DISA®. The frequency

500 Hz corresponding to lower time constants of about 100 and 10 milliseconds respectively. Routinely recording of the oscillatory potential was done using a frequency range of 70-500 Hz (see Fig. 1). Measurement of the amplitudes of the oscillatory potential was done according to the method of Smonsen (1961) thus obtaining a summed value of the amplitudes. The amplitudes of the different components were assessed by caliper square measurements. The error of measurement was estimated to $\pm 5\%$ this error being small in comparison with the recorded amplitude and the standard deviation of the mean.

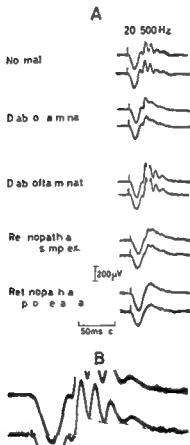


Fig. 1

shows different ERG responses to the stronger flash. Each ERG in the figure consists of superimposed responses. In each of the ERG-pairs the upper recording represents the right eye while the lower represents the left eye. The amplitudes as shown in B are the mutual distances between the peaks of the oscillations and the dotted lines connecting the troughs. Thus the value of the added amplitudes indicates the deviation from the imaginary smooth curve.

Table IV

The findings at the first follow up shown in Table III are split up in relation to type of diabetes at the initial investigation. The numbers in brackets denote the number of eyes initially presenting proliferative retinopathy at the initial investigation.

Duration of diabetes at the initial examination (in years)	Oscillatory potential	Ophthalmoscopic appearance (No. of eyes)		
		Unchanged retinopathy	Deteriorated	
			simple retinopathy	proliferative retinopathy
0-5	Hypernormal	7	0	0
	Normal	14	3	0
	Subnormal	3	1	0
6-10	Hypernormal	8	3	0
	Normal	11	9	0
	Subnormal	4	3	1
11-15	Hypernormal	13	0	0
	Normal	13	6	0
	Subnormal	3	0	1
16-20	Hypernormal	6	2	0
	Normal	3	6	3
	Subnormal	1	3	1
> 20	Hypernormal	6	0	0
	Normal	9	4	0
	Subnormal	0	2	6
Total No		103	48	8

Table VI shows that the transition to proliferative retinopathy has also taken place in those initially presenting hypernormal and normal oscillatory potentials regardless of the disease duration.

Deterioration in visual acuity could be demonstrated predominantly among the initially presenting subnormal oscillatory potential. At the first follow up (Table VII) none of the eyes initially presenting hypernormal values of the oscillatory potential showed changes in visual acuity. In eyes initially showing values of the oscillatory potential within the normal range only 8 out of 93 eyes (9%) had

veloped slight to moderate deterioration in visual acuity. However, in the group of eyes initially presenting subnormal values of the oscillatory potential no less than 2 out of 76 eyes (42%) had developed deterioration in visual acuity and 10 of these were legally blind ($P < 0.001$).

At the second follow up (Table VIII) only 2 out of 41 eyes (5%) initially presenting hypernormal values of the oscillatory potential had developed slight deterioration in visual acuity. Among eyes initially showing those of the oscillatory potential within the normal range 34 out of 106 eyes (32%) had diminished visual acuity, 13 of these being legally blind. In the group of eyes initially presenting subnormal values of the oscillatory potential no less than 11 out of 50 eyes (60%) had developed deterioration of the visual acuity, 29 of these being legally blind ($P < 0.001$). Only three patients initially presenting subnormal values of the oscillatory potential had developed slight cataract with visual acuity ranging from 19 to 6/24.

Table I

Relationship between the initial value of the oscillatory potential and the ophthalmoscopic fundus changes in 238 eyes of 194 patients at the second follow up. The numbers in brackets denote 38 eyes from 19 patients deceased during the follow up period.

Initial value of oscillatory potential	No	Diagnostic group	No
Hypernormal	31 (+6)	unchanged (\pm simple r)	20 (+6)
		deteriorated (simple r)	14 (+0)
		deteriorated (prolif r)	3 (+0)
Within normal range	46 (+10)	unchanged (\pm simple r)	38 (+2)
		deteriorated (simple r)	37 (+9)
		deteriorated (prolif r)	21 (+6)
<i>Subnormal or extinguished</i>			
\pm simple retinopathy	49 (+9)	unchanged (\pm simple r)	9 (+0)
		deteriorated (simple r)	18 (+1)
		deteriorated (prolif r)	28 (+8)
with proliferative retinopathy	18 (+13)	proliferative retinopathy	18 (+13)

Table VI

The findings at the second follow up shown in Table V are split up in relation to the degree of diabetes at the initial investigation. The numbers in brackets denote eyes free from proliferative retinopathy at the initial investigation.

Duration of diabetes at the initial examination (in years)	Oscillatory potential	Ophthalmoscopic appearance (No. of eyes)		
		Unchanged retinopathy	Detected	
			simple retinopathy	proliferative retinopathy
0-5	Hypernormal	3	4	9
	Normal	9	1	3
	Subnormal	1	4	1
6-10	Hypernormal	4	5	0
	Normal	3	6	9
	Subnormal	0	4	6
11-15	Hypernormal	8	3	0
	Normal	9	10	3
	Subnormal	1	5	10 (7)
16-20	Hypernormal	5	2	1
	Normal	4	9	11
	Subnormal	0	4	16 (10)
> 20	Hypernormal	6	0	0
	Normal	3	5	1
	Subnormal	1	3	3 (1)
Total No		63	63	146 (91)

Discussion

This represents the first prospective study made to estimate the prognostic value of the oscillatory potential in diabetic retinopathy.

After the initial recording of the oscillatory potential the patients were re-examined ophthalmoscopically and visual acuity recorded 6-8 years and 13-15 years later. 78% of the patients were reexamined 6-8 years after the initial investigation and 91% were reexamined 13 to 15 years after the initial investigation including the last known status of the decreased in the follow up period.

TABLE III

Visual performance of 908 eyes of 107 patients at the first follow-up. The numbers in brackets denote the percentage with oscillatory potential initially present with the following criteria:

Initial oscillatory potential	Initial visual acuity (1961-66)		Visual acuity 1972			
	Snellen value	n	Uncorrected		Corrected	
			6/9	6/12-6/18	<6/18	≤6/36
Hypernormal (n 47)	6/6	47	47			
Normal (n 85)	6/6	81	73	5	2	1
	6/9	3	3			
	6/12	0	0			
	6/18	1	1			
Subnormal (n 57 (+19))	6/6	55 (+10)	36 (+7)	12 (+2)	3	1
	6/9	1 (+7)	1 (+1)		0 (+0)	
	6/12	1 (+1)			1	
	6/18	0 (+1)			0 (+1)	

Table VIII

Visual performance of 38 eyes of 19 patients at the second follow up. The numbers in brackets denote those patients with subnormal oscillatory potential initially presenting with proliferative diabetic retinopathy

Initial oscillatory potential	Formal visual acuity (1961-66)		Visual acuity 1979			
	Snellen value	n	Unchanged	Deteriorated		
				6/9	1/12-6/18	<6/18-≤6/36
Hypernormal (n = 11)	6/6	11	11	2		
	7/7	10	22	11	1	1
	8/8	1				
	9/9	1				
	10/10	1				
	11/11	1				
	12/12	1				
	13/13	1				
	14/14	1				
	15/15	1				
	16/16	1				
	17/17	1				
	18/18	1				
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	92/92	1				
	93/93	1				
	94/94	1				
	95/95	1				
	96/96	1				
	97/97	1				
	98/98	1				
	99/99	1				
	100/100	1				

Neither the blood sugar level at the time of the investigation nor intravenous infusion of glucose was found to influence the size of the oscillations thus showing that short term dysregulation does not influence the oscillatory potential

Alterations in the oscillatory potential in diabetics without retinopathy must reflect functional changes in the neurophysiologic properties of the retina before ophthalmoscopic changes are detectable

The reexamination 6-8 years after the initial investigation confirmed the assumption that measurement of the oscillatory potential is of predictive value in juvenile diabetics since by this time 54% of those belonging to the subnormal group had developed proliferative retinopathy while only 6% of those in the normal range had developed proliferative retinopathy. If those who developed proliferative retinopathy during pregnancy are excluded only 2% of those in the normal range had developed proliferative retinopathy. None in the hypernormal group developed proliferative retinopathy.

The predictive value of the oscillatory potential is however restricted to those diabetics with a duration of the disease of more than five years since proliferative retinopathy could not be demonstrated 6-8 years later in those who had had diabetes for less than five years at the initial investigation.

The normal or hypernormal oscillatory potential therefore to a very great extent excludes the development of proliferative retinopathy within the next 6-8 years.

The reexamination 13-15 years after the initial investigation showed however that 7% in the hypernormal group, 26% in the normal range and 62% in the subnormal group had developed proliferative retinopathy.

The predictive value of the oscillatory potential is therefore mainly restricted to a period of 6-8 years in juvenile diabetics with a duration of diabetes of more than 5 years at the time of recording the oscillatory potential.

As shown in this material determination of visual acuity is of no predictive value in diabetic retinopathy. Retrospectively those developing reduced vision were mainly those presenting proliferative retinopathy at the initial investigation or those who had developed proliferative retinopathy in the observation period and predominantly those initially presenting reduced oscillatory potential.

Conclusion

Measurement of the oscillatory potential in juvenile diabetics with a disease duration of more than five years is of great predictive value in selecting those at risk of developing proliferative retinopathy.

The predictive value of the oscillatory potential can be extended to at least 6-8 years.

The predictive value of the oscillatory potential is doubtful in women who later become pregnant

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OSCILLATORY POTENTIAL AND NYCTOMETRY IN INSULIN DEPENDENT DIABETICS

BY

KIM FROST LARSEN¹ HANS WALTHER LARSEN and
SVEND ERIK SIMONSEN

The study draws a comparison between the oscillatory potential of the electroretinogram and the initial dark adaptation measured by nyctometry with the aim of assessing the predictive value of nyctometry in juvenile diabetics. The study included 61 insulin-dependent juvenile diabetics aged 18-49 years with a disease duration of more than five years. A statistically highly significant correlation could be demonstrated between alterations in the oscillatory potential and the initial dark adaptation. The results justify the assumption that nyctometry can be used as an easily handled clinical tool in selecting those at risk of developing proliferative retinopathy in their subsequent 6-8 years.

Key words: diabetic retinopathy - electroretinography - oscillatory potential - dark adaptation - nyctometry

The well-documented beneficial effect of photocoagulation on proliferative diabetic retinopathy is mainly restricted to early stages of neovascularization (Diabetic Retinopathy Study Research Group 1976 Hamilton et al 1976 Cheng et al 1978 Osterhuus et al 1979) making it crucial that the proliferative cases are detected as early as possible. Relying on ophthalmoscopy the current screening method

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incipient neovascularization may be missed and even the most thorough ophthalmoscopy does not exclude the development of neovascularization within a period. Proper detection of early proliferative retinopathy based on visible pathological changes therefore requires control examinations at an almost unpractical frequency. Therefore there is a need for a quick and easily handled screening test that will predict with reasonable certainty the course of a retinopathy in the individual diabetic for a number of years. Such a test would make it possible to identify patients who might develop proliferative diabetic retinopathy and follow them closely.

In 1962 Yonemura et al. showed that the so-called oscillatory potential, a small oscillating component of the human electroretinogram, was selectively reduced even in early stages of simple diabetic retinopathy. This finding was later confirmed by Simonsen (1965) who also found diminution in the oscillatory potential in diabetics without ophthalmoscopically visible fundus changes and suggested that the reduction of the amplitudes of the oscillatory potential reflected ophthalmoscopically invisible pathophysiological changes preceding the development of proliferative diabetic retinopathy (Simonsen 1968). In the belief that determination of the oscillatory potential in juvenile diabetics could predict the course of retinopathy in the individual case a prospective study on the prognostic value of the oscillatory potential was started in 1964. However even though this assumption was confirmed during the period of investigation (Simonsen 1970) we realized that proper recording of the oscillatory potential apart from being inconvenient to the patient is too time- and staff-consuming to be suitable for screening. In an endeavour to develop a simple test carrying the same predictive information as the oscillatory potential we recalled an article published by Gliem & Schulze (1970). In a group of 153 diabetics they found that with increasing severity of diabetic retinopathy there was a progressive deterioration in both the initial 2 min of dark adaptation and the ability to overcome a subsequent glare. Their results were obtained by nyctometry, a standardized quick and simple procedure for measuring the macular adaptation. For unknown reasons these authors have not further explored their original observations.

Interestingly the distribution of the results of initial adaptation in relation to the degree of retinopathy obtained by Gliem & Schulze (1970) showed a close resemblance to the distribution of Simonsen's electroretinographic results (Simonsen 1968). We assumed that such an apparent conformity could hardly be accidental but might rather be due to functional changes in the diabetic retina affecting both the initial course of dark adaptation and the generation of the oscillatory potential.

Therefore a comparative study of nyctometry and oscillatory potential was made in a group of insulin-dependent diabetics.

Material and Methods

The comparative study of the oscillatory potential and nyctometry was performed in 30 women and 31 men with insulin-dependent diabetes mellitus, the median age being 31.4 years. All were selected at random among the patients of the Seno Memorial Hospital. The duration of diabetes ranged from 1 to 15 years, the mean duration being 14.6 years. In all patients the disease had been diagnosed before the age of 30. All were treated with insulin and diet and all received insulin therapy. The daily insulin ranged from 0.33 to 1.54 IU/kg, the mean total dose being 0.85 IU/kg.

Primary exclusion criteria were arterial hypertension, nephropathy, visual acuity less than 6/9, refractive errors exceeding 3 dioptres, other than diabetic retinopathy, consumption of drugs known to influence the electroretinogram and pregnancy.

Prior to the actual admission every patient had for years been controlled at intervals of 3 to 6 months at the outpatient clinic. The main reason for the actual admission was a need for minor adjustment of the metabolic regulation.

The ophthalmological examination included refraction and corrected visual acuity, slit lamp examination, ophthalmoscopy, colour photographs of the fundus, fluorescence angiography, electroretinography (oscillatory potential) and nyctometry.

Prior to the study, electroretinography and nyctometry was performed on 10 eyes and 40 eyes respectively from healthy volunteers aged 23–47 years.

Nyctometry was performed on both eyes after about 1.5 min stay in the dimly illuminated examination room. The recording was done monocularly with the pupil unmedicated, the opposite eye being tightly covered with an eye pad in order to minimize interocular light adaptation. The recording was performed by means of the registration nyctometer (Registrier Nyktometer) originally developed by Comberg and manufactured by VEB Carl Zeiss (Jena, GDR). In the present design the registration nyctometer is equipped with a standard test programme which is automatically completed within only 6.5 min, thus making it suitable for group examinations. Nyctometry has gained wide application as an easy screening method for night blindness among military personnel and civilian lorry-drivers and for that purpose different authors independently have examined large numbers of healthy persons in order to settle normal values for different age groups, mono- and binocularly respectively (Lehnert & Schmidt 1966, Sack 1968, Bruschmann 1969, Patz 1969, Hepp et al. 1970). The patient looks through an optical system into a uniformly illuminated, red-coloured globe in which a dimmable sight test chart is placed in an opening just opposite the patient's eye (or eyes). The test signs are numbers in random order (5 in each line) and 10 lines of different sizes correspond to visual acuities ranging from 0.1 to 1.0. The order of the lines can be changed by the examiner outside the apparatus. The sight test chart is indirectly illuminated, giving a constant luminance of 0.5 asb (0.16 cd/m² (or nt)). By means of the optical system the test types are pictured in the patient's far point, thus making the test independent of the accommodation state. To the right of the sight test chart there is a red opening for the glaring light. The illuminance of the glaring light is 30 lux and its angle to the sight line 8 degrees. The test programme consists of three separate steps: 1) In the first 3 min the eye is adapted to a strong, uniformly white background illumination, the luminance being 2000 asb (about 2000 cd/m²). After this light adaptation period the light is switched off and 2) in the following 2 min the initial phase of dark adaptation (the instant adaptation) is recorded. In the dark globe only the faintly illuminated sight test chart is now visible. The patient is asked to read the test signs in the uppermost line (corresponding to a

visual acuity of 0.1) as soon as possible and when at least 4 of 5 optotypes are correctly to advance successively to the following line. The instant adaptation is thus recorded as increase in visual acuity as a function of time. 3) The last step is a measure of the sensitivity to glare in which the patient's increase in visual acuity is tested during exposure to constant glaring light. During the glare the luminance of the sight test chart increases successively from the standard (0.5 asb) to 4 (1.3 cd/m²) and finally to 32 asb (10 cd/m²) in intervals of 27 seconds. The examiner continuously transcribes the patient's reading on a rotating drum, thereby drawing an adaptation curve. In this was the initial amount of adaptation and the sensitivity to subsequent glaring are recorded graphically as an increase in visual acuity as a function of time. The recorded curve is finally compared to supernormal curves according to age, showing the normal ranges within the 2.5% and 97.5% percentiles. The different light sources of the apparatus were calibrated at regular intervals to ensure constant illumination.

Prior to the recording each patient had the procedure thoroughly explained since patient cooperation is essential for proper performance of the test.

Prior to electroretinography, fundus photography and fluorescein angiography the pupils were dilated with tropicamide (Mydracyl®) 0.5% and cyclopentolate (Cyclogyl®) 1%.

The oscillatory potential was determined shortly after nyctometry and recorded as described in Simonsen's study (1968). Recording was done on Kodak® film and the amplitude of the oscillatory potential was measured as the mean value obtained from 3 consecutive electroretinograms (Fig. 1). Due to the technical arrangement only one eye (always the left) could be examined.

Retinal fluorescein angiography was performed after the ERC 1 koma RC-Wide 42 (42) fundus camera equipped with Kodak W 17 and Kodak W 26 interference filtered excitation and background barrier was used. No adverse effects from fluorescein were recorded. All angiograms were assessed independently by the authors.

In each eye the following classification of retinal changes was used based on fundus photography and fluorescein angiography: 1) no retinopathy; 2) maximally 3 small aneurysms; 3) more than 3 microaneurysms with or without retinal haemorrhages, and additionally with hard exudates; 4) proliferative retinopathy with neovascularization in peripherally located; 5) proliferative retinopathy with preapillary neovascularization.

The ophthalmological examinations were carried out in one day.

In each case informed consent was obtained prior to the investigation.

The Spearman correlation coefficient was calculated in the statistical analysis of the data.

Results

The mean value (\bar{x}) of the oscillatory potentials obtained from the normal volunteers was 273 μ V, the sd being 21 μ V. In his comprehensive study Simonsen (1968) found a normal range ($\bar{x} \pm 2$ sd) of 223–317 μ V. The results of nyctometry from the normal volunteers all fell within the normal range.

The results of nyctometry were classified into three main groups: 1) subnormal nyctometry denoting values below the 2.5% percentile of the normal range; 2) values within the normal range; 3) hypernormal values exceeding the 97.5% percentile.

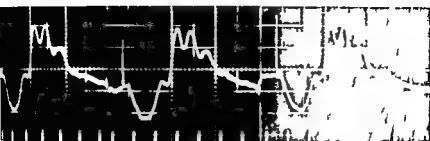


Fig 1

Oscillatory potentials from a 26 year old diabetic woman. The mean value of 3 consecutive electroretinograms corresponds to 110 μ V and the time scale denoted by the underlying

10
20

Tables I and II show the results of the comparative study. The results of nyctometry are divided into the three main groups. The oscillatory potential corresponding to these are shown in Table I. A comparison of the results of nyctometry is made to determine the separation in the mean values of the oscillatory potential groups. In the subnormal group performances with a mean value have been classified as severely reduced nyctometry. The results from subnormal results slightly below the lower limit of the distant division of the normal range the recorded values are in the low middle and high zones of the normal range.

10

Table I

The correlation between nyctometry and oscillatory potential (added amplitudes)

	No of eyes	Oscillatory potential (μ V)	
		$\bar{x} \pm SD$	Range
Nyctometry			
Reduced	25	71 ± 15	11-180
Normal	30	263 ± 9	170-380
Hypernormal	6	360 ± 11	230-400

The results of nyctometry are divided into three main groups. The mean values ($\bar{x} \pm SD$) of the amplitudes of the oscillatory potential corresponding to these groups are markedly separated ($P < 0.01$).

Table II

The correlation between nyctometry and oscillatory potential (OP) (added amplitudes)

	No of eyes	Oscillatory potential (μV)	
		$\bar{x} \pm SD$	Range
<i>Nyctometry</i>			
Severely reduced	18	28 ± 18	0-110
Subnormal	7	116 ± 9	0-180
<i>Normal range</i>			
Low	7	210 ± 8	110-310
Middle	13	256 ± 9	30-390
High	10	303 ± 13	20-380
Hypernormal	6	360 ± 21	255-405

Even if the results of nyctometry are further subdivided (see text) no overlapping exists between the corresponding mean values of the amplitudes of the oscillatory potential

Table III

The results of nyctometry ($n = 122$ eyes) in relation to the stages of diabetic retinopathy

Diagnostic group	Nyctometry					Hypernormal
	Severely reduced	Sub-normal	Normal range			
			Low	Middle	High	
No retinopathy			1	10	8	1
≤ 3 m.d.				11	4	0
> 3 m.d.						
± haemorrhages		2	7	8	1	
with hard exudates	1	11				
<i>Proliferative</i>						
Retinopathy						
peripherally	7	2				
prepapillary	20					

Severely reduced values of nyctometry are only found in advanced stages of the retinopathy

Analysis of the numeric values of the oscillatory potential and the nyctometry revealed a statistically significant correlation the correlation coefficient $r = 0.37$ corresponding to a P value less than 0.01.

When the values of nyctometry are compared to the degree of diabetic retinopathy in the same eyes (Table III) it is apparent that the eyes showing no or only slight retinopathy display normal nyctometry while severely reduced values of nyctometry are only found in advanced stages of diabetic retinopathy.

Discussion

Diminution or extinction of the oscillatory potential has been found to be a feature of advanced diabetic retinopathy. Moreover the oscillatory potential tends to diminish with increasing severity of retinopathy and finally to be extinguished in proliferative retinopathy (Simonsen 1965 Brunette & Desrochers 1970 Galloway et al 1972 Cysterberg 1974).

The demonstration of reduced values of the oscillatory potential not only in eyes presenting advanced diabetic retinopathy but also in eyes without or with only slight retinopathy indicates that electrophysiological disturbances precede ophthalmoscopically visible changes (Simonsen 1968).

Recording of the oscillatory potential in juvenile diabetics with a duration of the disease longer than 5 years is valuable for identification of those at risk of developing proliferative retinopathy. A normal oscillatory potential excludes with great certainty the development of proliferative retinopathy for at least 6 years. The predictive value of the oscillatory potential is probably more limited in diabetic women who later become pregnant (Simonsen 1980).

However recording of the oscillatory potential is time- and staff-consuming and therefore not as suitable for screening as nyctometry.

The present demonstration of progressively reduced initial dark adaptation in relation to increasing degrees of diabetic retinopathy is in accordance with the findings of Gliem & Schulze (1975). Reduction of the initial dark adaptation has also been reported in macular diseases (Batra & Paul 1967 Henkind & Siegel 1967 Severin et al 1967).

Dark adaptation is a complex function consisting of photochemical, neural receptor and network adaptation, the total resultant achievement being an optimized interaction of all three mechanisms (Virsu 1978). Although the mechanisms responsible for the increased recovery time in the initial phase of dark adaptation are not known, the phenomenon appears related to disturbances primarily in the neural network adaptation.

Examination of the total course of dark adaptation using e.g. the Goldmann-Weeker adaptometer has failed to show abnormalities even in advanced diabetic retinopathy (Cambie 1979). However this procedure does not permit a detailed study of the initial phase of dark adaptation.

The highly significant correlation between alterations in the oscillatory potential and the initial dark adaptation in diabetic retinopathy documented in this study suggests a common mechanism responsible for both the reduction of the oscillatory potential and for the initial phase of dark adaptation.

Microelectrode investigations in animal retinæ (Brown 1968) and clinical observations in man (Armington 1974) indicate that the oscillatory potential originates in the middle retinal layers especially in the inner nuclear layer (Wachtmeister & Dowling 1979). At present it is not exactly known which cell type acts as a generator of the oscillations.

The site of the neuronal mechanisms of the dark adaptation is likewise believed to be located to the inner nuclear layer (Dowling 1967). This might therefore also be influenced by the same functional disturbances which suppress the generation of the oscillatory potential. Therefore it is tempting to speculate that the reduction of the initial phase of the dark adaptation which can be measured by nyctometry reflects the functional counterpart of those early pathophysiological changes which may precede a transition into changes of proliferative diabetic retinopathy.

In two separate studies (Andersen et al. 1980, Frost Larsen et al. 1981) a significant improvement in the dark adaptation has been demonstrated in newly diagnosed juvenile diabetics after a 10-day period of superregulation in a biostator. Furthermore in a group of juvenile diabetics selected for close monitoring of the plasma glucose level and subsequent self adjustment of the daily insulin dose a significant improvement in nyctometry could be demonstrated in the patients achieving better long term (10-12 months) metabolic regulation. This indicates that in metabolic dysregulation the results of nyctometry are reversible to a certain extent provided the reduced values of nyctometry are due to functional changes in the retina. In cases without dysregulation of diabetes reduced values of nyctometry reflect irreversible functional changes in the retinae in due course probably producing progressive morphological changes which in most cases lead to proliferative retinopathy.

The significant correlation of the oscillatory potential and nyctometry in juvenile diabetics with a disease duration for more than five years justifies the assumption that nyctometry can be used as an easily handled clinical tool for identification of those at risk of developing proliferative retinopathy within the subsequent 5-10 years.

The probable predictive value of nyctometry indicated by this comparative study is being further investigated in a prospective study now in progress.

Acknowledgments

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OPHTHALMIC CHANGES FROM AGE OF 10 TO 18 YEARS

A longitudinal study of sequels to low birth weight I Refraction

BY

HANS C. FLEDELIUS

A report is given on refractive change (ΔR) from age of 10 to 18 years. The 1979 follow up comprises 137 persons who had earlier (around 1970) participated in a larger investigation into ophthalmic sequels to a low birth weight ($n = 599$).

In general (127 out of 137) there is a shift towards lower dioptric values (increase in refraction). Seven remained static while three showed a slight decrease in refraction (-0.25 to -0.50 D).

Adult emmetropia and hypermetropia show a median ΔR about 0.12 D (increase) during adolescence against median values of 1.1 and 2.5 D in juvenile myopia of ex-prematures and full-terms respectively. With a median ΔR of 1.2 D myopia of prematurity occupies an intermediate position (21 eyes of 13 subjects). Except for this subgroup there is no evidence that low birth weight has influenced refractive distribution.

With a (planned) skewing towards myopia, the 1979-sample cannot be considered epidemiologically representative as was the original 1970-material. Concerning the latter the 18-year incidence of myopia is given as 17.6% for ex-prematures and 13.1% for full-terms (*in minus figures based on school medical records*).

Key word: ophthalmic sequels - prematurity - low birth weight - longitudinal study - adolescence - refraction - juvenile myopia - myopia of prematurity - refractive change

Despite considerable interest in the functional and morphological development of the young eye there are few major projects in the literature that treat the subject. This is especially true as regards longitudinal studies of refractive and vision during childhood and adolescence.

The present study is meant to reduce this gap in the literature. Because of part of the children from an earlier cross-sectional study deals with refractive changes from the age of 10 to the age of 18 years (Fledelius 1976) and because further laid on the possible long term effects of a very low birth weight.

Material

The present follow up study comprises 13 18-year-old Danes who had earlier had an examination around the age of 10 as part of a larger scale investigation of the sequelae to a very low birth weight.

The sample derives from 'The University Hospital of Copenhagen' project 'The Significance of Gestation and Delivery for the Health and Development of the Child' designates a longitudinal paediatric study of 4000 pregnancies resulting in live births or abortions over 250 g (Zachau-Christiansen Thesis 1979).

In my 10-year study (Fledelius 1971) 30% of the low weighters (LBW) < 1000 g full term controls (FT-BW) 3-4000 g) attended for ophthalmological examination. The rates of those invited a representative picture was obtained of the two groups.

In 1979 a grant made it possible to re-examine part of the 139 children from the 1969-70 investigation (in the following called the 1970-study). In 1979 were invited to those where a follow up was considered of special interest as estimated from the 1970-study. The 1979-criteria were:

- 1) Both birth weight groups (< 1000 g and 3-4000 g) were to be fairly represented.
- 2) Among the ex-prematures emphasis was laid on the lower birthweighters.
- 3) All refractive groups should be included but while Emmetropes and hypermetropes were randomly sampled from the 1970-cases it was attempted to examine all cases of myopia at 1970-status or later developed with refractive error derived from medical records.
- 4) Again considering the myopes those with a past of prematurity were encouraged to attend.
- 5) For practical purposes those who had not the mean eye measured and (or) were Copenhagen were not included.

From this it is evident that the 1979-follow up does not truly reflect the original sample of the two basic birth weight groups from 1970. While the percentage of the original representativity has been lost. This should be kept in mind in the following.

Composition of the 1979-series

The low birth weight group (LBW) comprised 11 young Danes and there were 11 controls (FT). The number of reported ex-prematures was however less due to the exclusion of one case. This was a female with a rather advanced refractive error.

Table I

Birth weight distribution (and mean values) in the basic ex premature group of 1970 and in the fraction with a 1979 follow up

	Number of ex premature examined around 1970 (n = 309)	Number of ex premature with ophthalmic follow up 1979 (n = 10)
Birth weight < 1000 g	5	1
BW 1001 - 1250 g	21	3
BW 1251 - 1500 g	64	6
BW 1501 - 1750 g	93	17
BW 1751 - 1999 g	109	11
BW \geq 2000 g as co-twin or co-triplet to one from the above BW-classes	17	9
Mean age at examination	10.3	18.4
Mean birth weight	1663 \pm 283	1561 \pm 249

for plasma and myopia of prematurity (J No 519 8 Fledelius 19 61) Uveitis and phthisis
but explained axial eye measurements remote from physiological range and refractive
values could not be stated due to opaque media. Her data would weight unduly if entered
into the statistics of the morphologically and functionally normal eyes.

Selection of the lower birth weight classes (in 1979) is reflected by Table I which comprises
birth weight distribution of the ex premature examined 1970 and the 1979-81 BW group.

Selection according to refraction is shown by Fig 1. It is evident that the myopes from their
1979 0-percentages have been concentrated above representativity - due to inclusion into
the 1979 study of only a small proportion of the available hyper- and emmetropes.

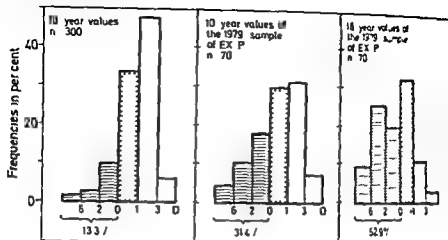
Methods

Cycloplegic refraction was measured by retinoscopy on both occasions. Subjective confirmation
was obtained with lenses in a trial frame and the customary 6-metre Snellen type. The
results are given as *pherical equivalent* i.e. the average between the refractive values of the
two main meridians.

The refractive change (ΔR) during the period was calculated as the 1970-value minus the
1979-value recorded with one decimal. This means that dioptric shifts towards myopia
increase in refraction i.e. given a positive sign.

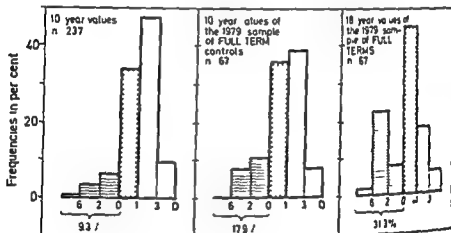
All reported eye findings are based on personal examination results. The exception is the
use of information from school medical records which served the purpose of tracing new

EX-PREMATURES



Refractive values (in Diopter)

FULL-TERMS



Refractive values (in Diopter)

Fig 1

Distribution of refractive classes of low birth weighters (top) and full terms (bottom). The selection of myopic cases for re-examination is apparent from the hatched areas. The diagrams show the total 10-year group (left) and the fraction with an 18-year follow-up (10-year values in the middle, 18-year values on the right). Emmetropic patients are indicated.

cases of myopia (because such cases were wanted for follow-up). The information is further utilized to give an estimate of the 18 year incidence of myopia in the (fairly healthy) low birth weight 1970-sample.

As part of the project other ophthalmic examinations were also performed. These are dealt with in separate publications which are in preparation.

Results

1. Incidence of myopia at age of 18 in the basic sample ($n = 539$)

According to the number of myopes re-examined and of other cases known to have developed myopia, the incidence of myopia around the age of 18 was found to be 11.6% for low birth weighters ($n = 302$) and 13.1% for full-termers ($n = 237$). These percentages represent minimum incidences because some cases may have escaped registration in the school medical records. In particular this holds true for those who left school at an early age.

The slightly higher incidence in low birth weighters is due to the 16 cases with myopia of prematurity. Excluding this fraction the incidence of ordinary juvenile myopia is almost similar in the two birth weight groups (12.3 and 13.1% respectively).

Exact incidences for emmetropia and low and high hypermetropia cannot be given. Those re-examined in 1979 (with such refractive values) are so few that they do not necessarily reflect what happened for the many who were not invited for the present follow-up.

2. Refractive change from age of 10 to 18 ($n = 137$)

127 out of 137 there was a shift towards lower dioptric values (increase in refraction). Seven remained static while only three showed a decrease in refraction (2.5 or 0.5 D).

Table II

Refractive change (towards myopia, in dioptres) from the age of 10 to 18 years. The material is subdivided by birth weight and sex. Mean values, standard deviation and range for right eyes only. Mean age at examination is also shown.

Sex	Number of persons	Refractive change in dioptres mean \pm SD	Refractive change in dioptres range	Mean age in years at exam	
				1970	1979
Low birth weight group					
M	36	1.17 ± 1.66	0-6.3	10.0	11.3
F	34	1.29 ± 1.03	0-4.0	9.8	11.2
Full-term control					
M	30	1.26 ± 1.23	-0.2-4.3	10	-
F	31	1.07 ± 0.91	-0.2-3.8	10	-

Table II shows the average change in refraction. A division by both sex and birth weight does not disclose subgroups that differ significantly from the rest. The results are based on individuals with right eye values only ($n = 137$). This is also based on the usually very high correlation between right and left eyes and the relevant regards to be taken in statistical evaluations (Ederer 1973, Flodius 1974).

The following Tables however do also comprise some left eyes ($n = 30$). These have been included due to significant anisometropia at first examination. Such differences are for instance a common feature of myopia of prematurity, probably because of varying degrees of damage (by the premature state alone or other extraneous influence). The two eyes are therefore regarded as somewhat far less dependent than usual and to augment information it is considered safe to employ data from both eyes.

Refractive change in refractive subgroups is given in Table III. Median values and 25-75% fractiles show that those with juvenile myopia (at 19 months) have undergone the most marked changes while emmetropic and hypermetropic have been rather static. Myopia of prematurity occupies an intermediate position.

The same features are evident from Tables IV and V where all eyes have been

Table III

Refractive change ΔR (in dioptres) from the age of 10 to 14 years in refractive subgroups (based on 19-month findings) of the two birth weight groups. Median value and 25-75 percentiles are shown. 137 right eyes are included - plus some left eyes ($n = 30$) deriving from individuals who exposed a significant anisometropia (more than 1 D) when last examined (1970).

	Number of eyes	Refractive change ΔR in dioptres median value	ΔR fractiles 25% - 75%
<i>Low birth weight group</i>			
Myopia of premat	21	1.2	0.1 - 2.1
Juvenile myopia	28	1.7	1.1 - 3.0
Emmetropia	26	0.7	0.2 - 1.2
Hypermetropia	11	1.0	0.2 - 1.9
<i>Full term controls</i>			
Juvenile myopia	23	2.3	1.3 - 3.3
Emmetropia	30	0.7	0.3 - 1.0
Hypermetropia	18	0.7	0.1 - 1.0

Table IV

Association between refractive change (ΔR from the age of 10 to 18 years) and final (adult) refractive class. Percentage distribution of four ΔR -classes on myopia (negative refractive values) emmetropia ($0 - +0.9 D$) and hypermetropia ($\geq +1 D$). Included are the same 157 eyes as in Table III.

	Refractive change (towards myopia) from age of 10 to 18			
	$\Delta R \leq 0.5 D$ n = 49	$\Delta R 0.6-1.5$ n = 61	$\Delta R 1.6-2.5$ n = 24	$\Delta R > 2.5 D$ n = 23
<i>1919 refractive classes</i>				
Myopia	25%	34%	71%	96%
Emmetropia	47%	44%	21%	4%
Hypermetropia	28%	22%	8%	
Total	100%	100%	100%	100%

are however pooled. Further pooling of birth weight groups seems justified from the two former tables which do not indicate significant differences associated with birth weight grouping (except the minor fraction with myopia of prematurity). Refractive changes below 1.5 D predominate in the subgroups of adult emmetropia and hypermetropia. Conversely, the more conspicuous refractive changes above 1.5 D are mainly confined to the myopes.

Table VI shows trends within the group of myopia. In 62% of those with myopia of prematurity (13/21 eyes) there has been only a slight increase in refraction.

Table V

Numerical distribution of the four ΔR -classes (cf. Table IV) on six, more specified refractive groups. Again n = 157 eyes.

	Refractive change (towards myopia) from age of 10 to 18			
	$\Delta R \leq 0.5 D$	$\Delta R 0.6-1.5$	$\Delta R 1.6-2.5$	$\Delta R > 2.5 D$
<i>1919 refractive values</i>				
$\geq +3 D$	1	6		
$+1$ to $+2.9$	13	-	2	
0 to $+0.9$	23	27	2	1
-2 to -0.1	7	11	4	
-6 to -2.1	3	9	8	18
$\leq -6 D$	2	1	2	4

Table VI

Refractive change in myopia of prematurity (left $n = 21$ eyes) and in the normal population with myopia (according to 1979-findings 1 BW + FT $n = 51$ eyes). As in Table IV and V, right eyes are included except anisometric eye pairs where the refractive

	Refractive change between age of 10 and 14 years in					
	eyes with myopia of prematurity ($n = 21$)			eyes with idiopathic myopia ($n = 51$)		
	ΔR $\leq 0.5 D$	ΔR $0.6-1.5$	ΔR > 1.5	ΔR $\leq 0.5 D$	ΔR $0.6-1.5$	ΔR > 1.5
Myopic subgroups accord to 1979 refraction values						
-2 to -0.1 D	3			4	11	1
-6 to -2.1 D	2	3	4	1	4	11
below -6 D	2	1	4			3

(below 1.5 D) during adolescence contrasting with 39% (20 of 51) in juvenile myopia. The clear difference between distributions however is not significant and is probably to be explained by the rather small size of the samples.

Finally, a short comment on corneal astigmatism as assessed by keratometry. The dioptric differences between main corneal meridians ranged from zero to 1 D. The mean value was 0.86 D and the median value 0.5 D. A change between 1979 and 1979 measurements was virtually absent. At least it cannot be stated whether the small differences actually found are due to real alterations or to errors of measurement.

Discussion

The age period from 10 to 18 years is interesting also for ophthalmologists.

For instance, it is within this period that many develop myopia and have to cope with optical obstacles for the first time in life.

Considering the otherwise stormy bodily changes around puberty, it is somewhat equally dazzling that a much higher proportion of eyes hardly seem to be affected. In fact, Sorsby et al. (1961, 1970) regard the eye as already fully grown around the age of 13. A permanent ocular state should thus be reached rather early and sexual maturation and growth in general are still far from full development.

From an epidemiological point of view eye clinic material has to be disregarded when assessing refractive change during adolescence due to self selection by attendance. Most valid information has been obtained from cross sectional studies many of which were briefly reviewed in chapter five of my Thesis (Fledelius 1976).

Several of these suffer from the drawback that they have focused on myopia leaving the important major fraction of children without visual trouble remain unstudied. This somewhat neglected part of the refractive spectrum is dealt with recently in cross sectional studies by Johnson et al (1979) and Laitinen & Lakkala (1980) and also earlier in my own series (Fledelius 1976).

Our present status is in brief: the bulk of school children have refractive values between zero and +2 D and the proportion of myopic cases shows an increase with advancing age. This reflects the general trend during adolescence: a shift towards lower dioptric values (increase in refraction). The exception is made up by high hypermetropia which is often found to be static.

The few published longitudinal studies mainly support this view: Hirsch (1952, 1961, 1964) followed Californian children (Ojai series) from school entry to the age of 14 and Sorsby & Leary (1970) reported on ophthalmic re-examinations performed during school age.

Using a so-called physiological method of relaxing accommodation (i.e. without cycloplegic eyedrops) a skewing towards lower refractive values is to be expected in Hirsch's series. Accordingly there seems to be too few cases of hypermetropia $\geq +1$ D (39% at school start, about 7.5% at the age of 13-14). Further several cases of accommodative pseudomyopia are probably contributing to the high 14 year incidence (18.3%) of low myopia (0 to -1 D).

Concerning Sorsby's series a certain degree of self selection made the representation dubious (as claimed by the author himself). For obvious reasons those who need glasses benefit more when volunteering for re-examination: this implies a skewing towards (myopic) cases with refractive changes above average.

Despite such objections a rough agreement between materials is evident. The prototype shows only a small increase (ΔR below 1.2 D) in refraction during school years: a minor fraction undergo more marked changes (above 1.2 D in 12% in Hirsch series and 28% in Sorsby's apparently skewed sample).

The present material fulfils one of the above requirements: that of being examined under cycloplegia on both occasions (after topical cyclopentolate 1% plus tropicamide 1%).

Due to selection criteria however a truly representative follow up picture cannot be given for all 339 who attended for 10 year assessment around 1970. In particular I have only gained incomplete information about those who end up with hypermetropia and emmetropia.

The present sample is with purpose even more skewed towards myopia than

that of Sorsby & Leary (1970). However it is not unreasonable to assume that the refractive changes of the three present 15 year groups (if myopia, presbyopia and hypermetropia) by and large reflect the true picture in the corresponding refractive groups of a young adult North western European population.

Another question is left unsolved. We do not know exactly when the refractive eye becomes static. This would require an interim eye assessment (at least) at an age of 15. We just know that some eyes have also shown changes after that, namely developing late juvenile myopia.

Eyes with myopia of prematurity make up a (minor) subgroup of eyes of damage (faulty development, Fledelius 1976) connected with the very low weight. Estimating from total refractive distribution however the low birth weight group does not otherwise seem to deviate from the full term curve. The controversial question will be further dealt with through analysis of other epidemiologic parameters in future publications.

Acknowledgment

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DEMONSTRATION OF A DIFFUSIONAL PATHWAY BETWEEN THE SUBRETINAL SPACE AND THE JUXTAPAPILLARY CONNECTIVE TISSUE

An In Vitro Experiment Using Horseradish Peroxidase as a Tracer

BY

TOR FLÄGE and ANDRÉ FLÄGE OLD

A defect in the blood-retina barrier in the optic nerve head region has been demonstrated. In order to show the exact localization of this defect, an in vitro experiment has been performed on rabbit eyes, using horseradish peroxidase as a tracer. From a subretinal deposit the tracer diffused between the innermost retinal pigment epithelial cell and the cells of the innermost intermediate tissue into the border tissue of Elschnig and the juxtapapillary choroid.

The defect in the permeability barrier is thus located to the junction between the retinal pigment epithelium and the innermost intermediate tissue. The role of the junctional complexes in the innermost intermediate tissue in relation to the blood-retina barrier is discussed.

Key words: blood-retina barrier - optic nerve head - subretinal space - retinal detachment - horseradish peroxidase - rabbit eye

After intravenous administration of horseradish peroxidase (HRP) the tracer leaked from the peripapillary choroidal capillaries into the juxtapapillary sensory retina in rabbit and monkey (Fläge 1980). This finding demonstrated a defect in the

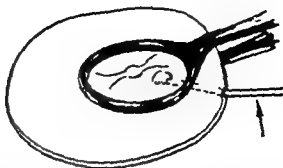


Fig 1

Schematic illustration of the experimental set up. A modified chalcid forceps, used in the posterior plate for the optic nerve, was used to isolate the central part of the rabbit eye. A small subretinal depot of HRP solution was made by careful injection of polyethylene tube (arrow). Following the injection the forceps was thinned around the tube.

blood reuna barrier in this region. The localization of the defect was supposed to be either in the juxtapapillary reunal pigment epithelium (RPE) in the fundus, in the intermediary tissue (KIT) or in the connection between these structures.

The present study was started in order to define the localization of the defect in the permeability barrier more precisely. The problem was tested in an animal experiment by studying the diffusion of HRP from a subretinal depot near the optic nerve.

Material and Methods

Eyes from five adult albino rabbits, which were killed with an overdose of pentobarbital sodium, were used in these experiments. The eyes were immediately enucleated and placed in sterile Ringer solution at room temperature. Then, the eye was opened and a modified chalcid forceps was used to isolate the central part of the posterior eye plate. The diameter of the ring on the forceps was 1.1 mm. In the whole plate a hole with a diameter of 3.5 mm was made for the optic nerve. By careful dissection from the equator a small polyethylene tube (Intramedic Clay Adams, outer diameter 1.0 mm) was inserted into the subretinal space. The tube was connected to a 1:1 syringe with a thin needle (outer diameter 0.37 mm) fitting into its opening. (The needle was 11 cm long and 0.37 mm diameter). Ringer solution was carefully injected to raise the tube (diameter) around the end of the tube. Great care was taken to avoid damage to the retina.

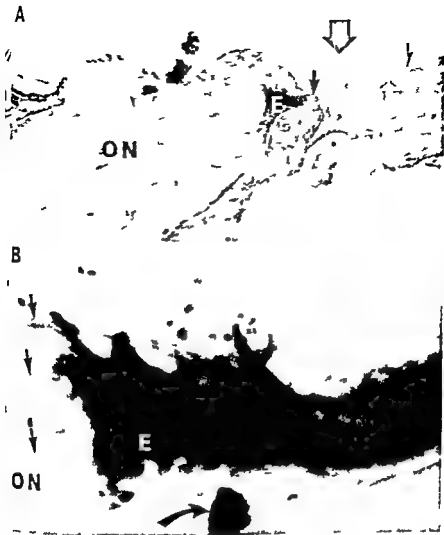


Fig 2A

stained cryostat section of the ONH region. The peripheral retina does not show any
 in of detachment. Broad staining indicates HRP activity. The peripheral retina facing the
 net deeper (broad arrow) shows distinct staining located in the outer part of the sensory
 retina (small arrows), the inner part of the border tissue of the ONH (broad arrow) and the
 choroid (dashed arrow). The HRP activity in the border tissue appears rapidly
 from the ONH. Optic nerve myelin is unstained. The outer retinal layers on the
 side of the ONH show faint staining. Broad staining is also seen in the outer layers
 present both in the ONH and in all parts of the border tissue (ON) (broad arrow).

Fig 2B

From Fig 2A. Fracture of the outer part of the border tissue of the ONH (broad arrow) shows
 staining of HRP activity in the adjacent border tissue (ON) (small
 arrow). The staining is present in the border tissue and in the outer layers of the
 retina (broad arrow).

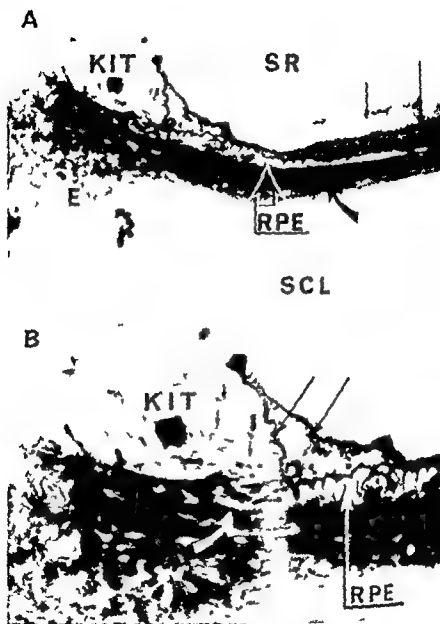


Fig 3A

Unstained light microscopic section from epim embedded tissue HRP in the subretinal space (SR) is to a large extent located to the rod cone layer (long arrow) while some substance have penetrated the outer limiting membrane and reached a level between the outer nuclear layer and outer plexiform layer (small arrow). The retinal pigment epithelium (RPE) is unstained by the tracer (open arrow) while the anterior part of the choroid (Elschnig's) and the juxtapapillary choroid (arched arrow) are distinctly stained. Intermediate tissue (KIT) Sclera (SCL) $\times 400$

towards the optic nerve head (ONH). Following the injection the forceps was held
 opened to close and immobilize the tube. The complete preparation was kept in the
 the Ringer solution at room temperature during the mounting process and for a
 varying diffusion time for the tracer following the injection. Diffusion times
 from the injection of the tracer until the preparation was immersed in liquid nitrogen
 of this time the whole preparation with the tube and the forceps were placed in
 precooled glutaraldehyde fixative (2.5% in 0.1 M phosphate buffer, pH 7.4). The
 times of diffusion times and tracer solution concentration were varied at
 5% the times ranging from 10 to 30 min and 1% solution of
 after glutaraldehyde fixation for 17 h the tissues were washed
 (pH 7.4) with 5% sucrose before being cut on a freezing
 10 μ m thick. The sections were incubated in tris HCl buffer
 solution (pH 7.6) for 40 min. Some of the thin sections were mo-
 stained without further staining. The rest of the sections were
 0.1 M phosphate buffer) dehydrated in graded ethanol and cleared
 microscopic sections of about 1 μ m thickness were examined with
 toluidine blue. The ultrathin sections were made with an LKB
 a Siemens Elmiskop 1A either unstained or after staining
 lead.

Results

light microscopy of unstained cryostat sections did
 xanthapillary retinal detachment (Fig. 2A). The juxtapapillary
 of tracer showed distinct staining indicating HRP activity in periretinal
 part of Elschnig border tissue but also in the adjacent choroidal
 layers. A striking feature was that the HRP activity in
 rapidly away from the ONH while the staining of the retinal
 tracer depot. Strands of HRP stained connective tissue protruded
 heavily stained anterior part of Elschnig border tissue into the adja-
 cent (Fig. 2B). On that side of the ONH opposite to the depot, the outer re-
 tina always revealed only faint staining and the choro did not show extrare-
 tinal HRP at all. The inner part of the sensory retina did not show HRP activity in an
 region. Staining due to intravascular erythrocytes was present both in the opti-
 c nerve head and in all parts of the choro d.

Fig. 3B

Detail from Fig. 3A. Long arrows point to HRP in the juxtapapillary subretinal space. In the
 adjacent IRT thin lines of HRP activity are seen (small arrows). That part of Bruch's
 membrane continuing towards the optic nerve beyond the junction between the RPE and the
 IRT (curved arrow) is heavily stained. The juxtapapillary RPE-cells are unstained by the
 tracer $\times 500$.

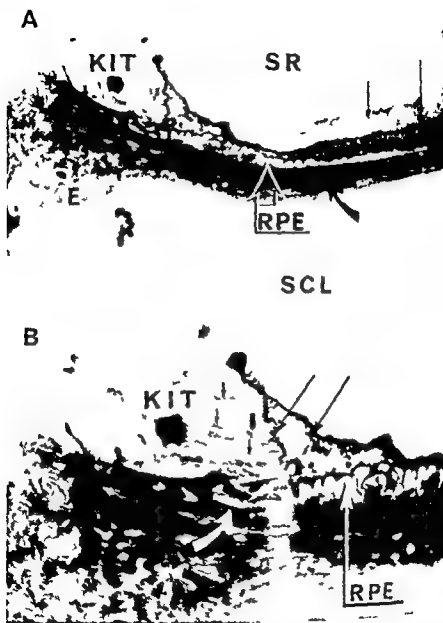


Fig 34

Unstrained light microscopic section from epon embedded tissue. HRP in the sensory retina (SR) is to a large extent located to the rod cone layer (long arrow) while some of the substance have penetrated the outer limiting membrane and reached a level between the outer nuclear layer and outer plexiform layer (small arrow). The retinal pigment epithelium (RPE) is unstained by the tracer (open arrow) while the anterior part of the Elkschnig (E) and the juxtapapillary choroid (arched arrow) are distinctly stained. KIT = intermediary tissue (KIT). SCL = sclera (SCL) $\times 500$

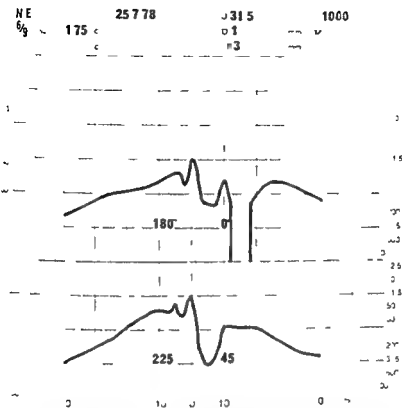


Fig 4

line penmetry profiles recorded in Case NE with active central serous retinopathy. Also plotted on the horizontal meridian. Below on the axis passing through the site of focal breakdown of the choroido-retinal barrier and the fovea.

in this case on the 180-0 axis and although the density of the defects varied from individual to individual these were always found to be larger and deeper when the axis selected for investigation corresponded to that joining the site of focal choroido-retinal barrier disturbances to the fovea (Fig 4). As the serous fluid absorbed paracentral scotomata became smaller and foveal sensitivity recovered in these cases (Fig 5).

After the detachment had settled 11 of the 14 patients with a recently active central serous retinopathy and 7 of the 13 cases with resolved central serous retinopathy of longer duration were noted to have needle shaped scotomata in the paracentral region. Scotomata varied between 1 and 3 in width and from a slight depression on static profile to quite dense cuts which approached the baseline of the chart.

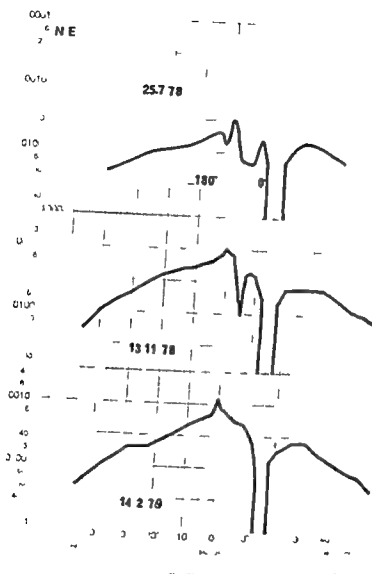


Fig 5

Recovery pattern of static perimetry profile of case NE over a period of 1 month

In 11 of these cases residual paracentral scotomata were detected on the axis. However 6 others showed no abnormality on this meridian but such became clearly evident if the static profiles were performed on the axis just site of the now sealed breach in the choroido-retinal barrier to the fovea

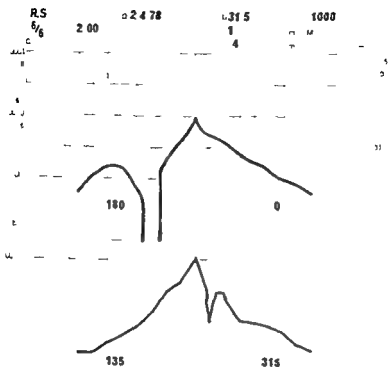


Fig 6

Static perimetry profile recorded 6 months after the onset of symptoms Case RS Normal field plotted on 180-0 axis Residual paracentral depression present at 135-315 axis

Needle shaped scotomata were found in 63% of cases with resolved idiopathic central serous retinopathy

The time taken for the serous fluid to absorb in the cases of central serous retinopathy examined in this study ranged from 2 to 11 months with a mean of 4 months. Of the 11 patients in whom detachments were observed to have resolved in less than 4 months 6 drew defects on Amsler grid and 3 of these noted to have static perimetric defects

In 16 patients whose detachments took 4 months or more to settle all 16 drew distortions on the Amsler grid tests and 14 retained abnormal static perimetric profiles. No patients were found to have residual changes on static perimetric examination who did not record defects on Amsler charting

Discussion

The results of this study clearly indicate that Amsler testing, which can be carried out quickly without recourse to any special technical expertise, is as good a static perimetry for evaluating central field disturbances in the acute phase of idiopathic central serous retinopathy and in some ways superior to static perimetry in assessing the incidence of residual central field defects since 81% of patients continued to plot distortions on the Amsler grid as opposed to 63% who retained clearly abnormal static perimetric profiles. The percentage of patients who continued to draw defects on the Amsler grid examined in our study is similar to that reported by Klein et al. (1974) who found that 89% of their patients required changes. The higher number of patients who drew distortions on the Amsler chart may well arise since it is possible by this method to plot changes associated with micropsia and metamorphopsia. Thus it would appear that the two forms of examination do not provide exactly comparable information about retinal function in patients who have developed a central serous retinopathy after the serous fluid has been absorbed since static perimetry testing detects predominantly the paramacular lesions where as Amsler charting often indicates more widespread changes at the posterior pole.

The greatest depression on static perimetric profiles is found not to coincide with the point at which a breakdown of the choroidal retinal barrier developed in the acute phase, a site which might be considered likely to show disturbances of retinal function but was invariably located pericentrally and most apparent with the axis which joined the foveal point to fixation. It would seem probable therefore on the basis of these findings and the shape of the scotoma detected on the Amsler chart in the acute phase that fluid accumulating in the space situated between the pigment epithelium and the neural fibres immediately over the break in the choroidal retinal barrier tracks towards fixation and that part of the retinal disturbances are caused by persistent malfunction of elements within the macula area.

Hatch et al. (1972) thought that the incidence of residual defects of retinal function in patients who had required a central serous retinopathy depended on how quickly the fluid was absorbed. The results of this study confirmed this impression as disturbances of the entire visual fields were found in 27% of patients where the serous fluid cleared in 1-3 months but rose to 85% in cases in which the apposition of the neural fibres of the retina to the pigment epithelium was delayed beyond that time. As Chouhury et al. (1973) showed that the level of recovery of central visual function in patients with successfully treated rhegmatous detachments involving the macula is related to the duration of retinal separation it would appear that similar but more subtle changes develop predominantly in the

and remain as a permanent feature in the majority of cases. The central serous retinopathy support is provided by the observations of Kroll & Machemer (1970) who, in their experimental studies, reported that cone outer segments appeared shorter and less well than their rod counterparts when experimentally induced detachments were reattached, and by the fact that patients with central serous retinopathy continue to score poorly on the Farnsworth Munsell 100 Hue Test (Leaver & Williams 1970).

Although in the majority of patients who have developed a central serous retinopathy the residual field changes cause little difficulty in continuing a high level of central visual function, the visual impairment in graphic or micro-engineering industries may remain permanent and in the execution of their work, and this study illustrates one of the reasons as to why, if these the disease process is not as benign as has previously been thought.

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COMPUTERIZED VISUAL FIELD SCREENING IN THE MANAGEMENT OF PATIENTS WITH OCULAR HYPERTENSION

BY

KJELL DYSTER AAS, ANDERS HEIJL and LEIF LUNDQVIST

Visual field testing with the Competer fully automatic computerized perimeter (Heijl & Krakau 1975) employing a supra liminal screening test procedure was used in a material of 1013 eyes with ocular hypertension in which earlier routine perimetry (kinetic and static) on the Goldmann perimeter had yielded a normal result. The automatic screening was repeated if positive and manual control perimetry was used in order to confirm or reject identified field defects. This procedure revealed field defects that could be confirmed at both automatic and manual perimetry in 3.6% of the eyes. In a control group the incidence of field defects found at manual perimetry during the same time interval was calculated at 0.4%. Thus automatic screening revealed several times more field defects than manual routine perimetry. Eyes in which repeated automatic screening had indicated defects which manual control perimetry failed to confirm showed a high percentage of field loss at later follow up. The results are discussed and the conclusion is drawn that automatic screening is clearly superior to manual routine perimetry used at present. The most practical solution in many eye departments would be to use a computerized perimeter for the visual field screening of glaucoma suspects.

Key words: visual field screening - automatic perimetry - computerized perimetry - ocular hypertension - glaucoma

The disclosure of a glaucomatous visual field defect in an eye with increased intraocular pressure immediately leads to a re-classification of the eye. It can no longer be labelled a hypertensive eye but has to be classified as a glaucomatous eye. This involves different management, maybe even surgery. The examination of the visual field can be considered the crucial part in the management of ocular hypertension. The task of checking the fields of such patients is difficult in several respects. First we are dealing with a very large number of patients, secondly the role of the perimetrist is not made any easier by the knowledge that all previous fields in the eye have been normal and that in ocular hypertension the likelihood that field defects may develop is small (Armaly 1979, Nelleman, Sørensen, Nielsen & Nørskov 1978, Linner 1976, Kitazawa et al. 1977, Perkins 1973). In such cases it might be an advantage if the field screening could be handled by an automatic perimeter. Much time and effort could be saved in such a way. There are indeed data available which indicate that automatic perimetry might work just as well as conventional manual perimetry in the detection of glaucomatous field loss (e.g. Heijl 1976, Heijl et al. 1980, Aulhorn & Durst 1977, Keltner et al. 1979). However one might go even further and suspect that in fact automatic screening perimetry might be superior to routine manual perimetry as it is usually performed in the detection of field defects in this group of eyes. A few earlier studies (e.g. Heijl 1976, Spahr et al. 1978) indicated that this might be true.

The purpose of this study was to analyse the results of the application of an automatic perimeter (the Competer) (Heijl & Krakau 1975) to a large material of eyes with increased intraocular pressure but normal fields i.e. eyes with ocular hypertension. We suspected that many field defects had been overlooked in these eyes.

Material

Two groups of patients were studied

- a) A test group of 1013 eyes in 541 consecutive patients. All these eyes were classified as ocular hypertensives. They showed or had shown elevated intraocular pressure (≥ 22 mm Hg) but normal fields at earlier routine kinetic perimetry on the Goldmann perimeter. The average time that had passed since the previous manual perimetry was 11.5 months (\pm SD 4.6 months).
- b) A control group of another 201 eyes in 115 patients. Also these eyes had ocular hypertension.

The average ages of the patients in groups a and b were 66 years (range 20-93 years) and 64 years (range 31-88 years) respectively.

STIMULUS	RESPONSE	CHOICE	MODE OF TEST	TIME	TIME	TIME
1	2	3	4	5	6	7
0	2	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	1	0	0	0	0	0
5	2	0	0	0	0	0
6	20	0	0	0	0	0
7	2	0	0	0	0	0
8	0	0	0	0	0	0
9	3	0	0	0	0	0
10	1	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
16	0	0	0	0	0	0



Fig 1

Computer print-out of a normal test result. The test result at each of the 64 test points is printed out numerically. The table (top left) contains the frequency distribution of the result of the 64 points. The left column shows the stimulus intensity number. A higher number means a higher sensitivity. Intensity level 0 denotes no response to the maximum stimulus. 9 a fairly low threshold. The right column indicates the number of the 64 points tested whose test results had the varying intensity levels. The Q value (6 here) is the most common value. Test points with a threshold equal to Q are printed out as 0 in the graph display. All points with a higher threshold are printed out as negative numbers and terms are added to negative numbers < -1 in order to make them stand out more clearly graphically. e.g. -6000 in the blind spot (compare Krakau 1978). Actual threshold determinations have been performed only at the initial four test points (marked in the print-out) and at missed points (in the blind spot).

Methods

Automatic perimetry

Perimeter

The test group was subjected to automatic screening perimetry on the Competer automatic perimeter.

The Competer perimeter (Heijl & Krakau 1975) is a fully automatic computerized perimeter. It has 64 static test points (light emitting diodes) concentrated in the central visual field from 5 to 20 from fixation (Fig. 1). The light emitting diodes can be lit at 16 intensity levels the ratio between two consecutive levels being 2:1. The test result is displayed by a printer as illustrated in Fig. 1.

Throughout the study we used 0.3 sec stimulus presentation time, an interstimulus interval of 2.0 sec, and a background illumination of 1.0 or sometimes 11.1 cd/m².

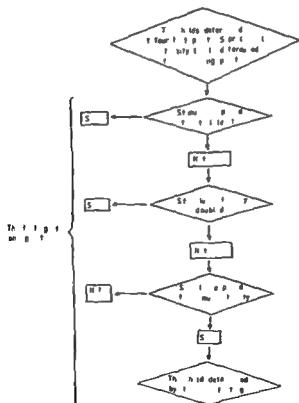


Fig 2

Test logic (simplified) All points are tested in random sequence. The figure shows the testing at one point.

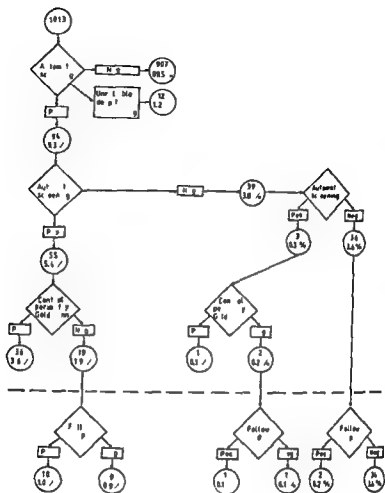


Fig 3
Flow diagram of study design (see methods and results)

Test logic

The test logic governing the test procedure was almost identical with that described by Heijl 1976. It is a screening test logic employing mainly supra-threshold stimuli. Its main features appear from Fig 2. Test time is 3-4 min per eye.

Interpretation

The interpretation of the automatic fields followed the criteria stated by Heijl 1976. Thus a visual field was considered normal if all points (outside the blind area) ended on a sensitivity level not more than one intensity level lower than the first intensity level at which it was exposed (cf Fig 1). One single test point outside the blind spot area with a lower sensitivity than that was enough to classify an automatic field chart as abnormal. In the Computer the patient's first

checked by the presentation at random intervals of stimuli in the blind spot area of the eye tested. Among these stimuli the number that has been perceived is divided by the total number of exposed stimuli. This quotient is denoted the fixation quotient. Automatic fields with a fixation quotient ≥ 0.25 were labelled unreliable and the test was repeated. If the fixation quotient at the repeated automatic screening was still ≥ 0.25 no further automatic perimetry was performed and the eye was excluded from the study (Fig. 3). Another criterion that had to be fulfilled for a test to be regarded as reliable was that the blind spot had to be present in the automatic chart. Otherwise the test was repeated just as in the case of a high fixation quotient.

Manual perimetry

Procedure

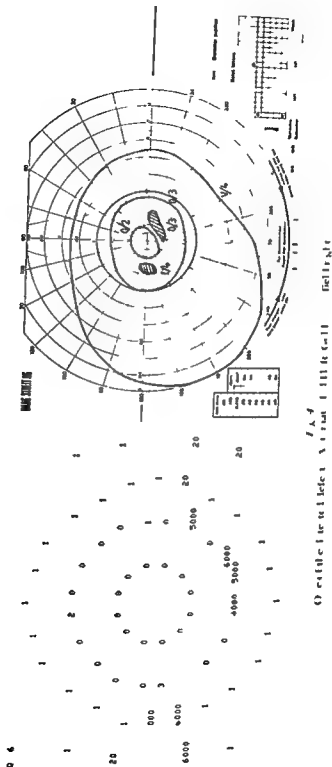
The manual fields were plotted with a combined static and kinetic procedure. At least two isopters were plotted: one peripheral and one as close to the 25° circle as possible. The central field from 2° to 15° was then tested statically with the same object as that of the 25° isopter in about 20 static spot checks.

Interpretation of Goldmann fields

The interpretation of the manual kinetic fields was done in the conventional manner. Nasal steps, arcuate lesions and paracentral scotomas were all considered abnormal, as was any distinct deviation of an isopter from its expected course. An isolated enlargement of the blind spot or a hazing of the blind spot with no other concomitant plottable defect was not taken as a sign of abnormality.

Final classification of eyes tested on the automatic perimeter

The classification of the eyes subjected to automatic perimetry is illustrated in Fig. 3. A combination of repeated automatic screening and manual control perimetry was used in order to confirm or reject defects found at the primary automatic screening of the 1013 eyes involved. Eyes with a *normal* test result at the initial automatic screening were not examined further. Eyes with an *abnormal* result at first screening were subjected to a second automatic test. If at this second screening a defect was found in the same area as in the first screening the eye was denoted as having a 'field defect confirmed at automatic perimetry'. This required that the first and the second test showed at least one common abnormal test point or that neighbouring points were abnormal in the first and second screening respectively. If this condition was fulfilled the eye which had thus demonstrated a field defect confirmed by automatic perimetry was subjected to manual control perimetry (Fig. 3). If this condition was *not* fulfilled a third automatic screening was performed, the result of which determined whether manual control perimetry should be carried out (cf. Fig. 3).



Control group

Since in the study group (a in Material) automatic screening was not performed at the same time as the routine perimetry on the Goldmann perimeter but on an average 11.5 months later we had to estimate the normal incidence of glaucomatous field loss in our material of ocular hypertensives when routine manual perimetry was performed. Automatic screening has come to be the routine method at our glaucoma unit for the screening of suspect glaucomatous field since its introduction in 1977. Therefore we did a retrospective study of 201 consecutive eyes with ocular hypertension which in 1974 had shown a normal result at routine kinetic perimetry. The result of the subsequent routine manual field test in these eyes was checked.

Results

Test group

Fifty five eyes (5.4%) showed an abnormal result at the two initial automatic screenings. Out of these 36 (3.6%) could be confirmed as pathological at manual control perimetry (field defects confirmed at automatic and manual perimetry) while in 19 no defects could be found at manual control perimetry. These latter 19 eyes thus represented the tentative false positives (1.9%) after automatic screening performed twice. However in this group more than half (10 eyes - 1.0%) showed reproducible field defects at later follow up in the same area as that indicated by the initial automatic screenings (Fig. 3 and Discussion).

There were 39 eyes (3.8%) where an initial positive automatic screening was followed by a negative automatic rescreening. Among these eyes three were positive at a third automatic screening, two of which later proved to have field defects (Fig. 3). Thirty six eyes were negative at this third automatic screening. Only two of these showed field defects on later follow up.

Control group

One out of the 201 eyes with normal fields at routine manual perimetry with the Goldmann perimeter in 1974 showed field defects at the next field test. The average time between these two tests was 14.2 months (\pm SD 4.4 months). When the routine perimetry was used this gave an incidence of field defects in our material of 0.5% in 14.2 months or 0.4% in the 11.5 months which was the average time interval between the manual routine perimetry and the initial automatic screening in the study group.

Discussion

If we subtract the incidence of field defects in the control group (five percentage of identified previously unknown field defects confirmed by automatic and manual perimetry in the test group (3.6–0.4%) we arrive at the figure. This represents a conservative approximation of the number of abnormal fields which would have been missed by routine manual perimetry as described before. This figure is too low for at least two reasons. First the assumption that automatic screening identifies *all* defects is not correct (e.g. Heyl et al. 1980). This is valid for all perimetric techniques. Secondly it seems quite clear that many of the new field defects confirmed at automatic but not at manual control perimetry really are true field defects at this time, since 53% (10 out of 19) at follow up 12 years later showed field defects in the area indicated by the first automatic screening. These defects were usually confirmed at automatic perimetry and were also found by manual perimetry in those cases in which this was performed. We then arrive at a figure of 4.6% detected fresh defects. This is a high figure considering the fact that the incidence of field defects in the material studied should be small – since hypertension is much more common than glaucoma with field defects. Certainly this figure is influenced to a certain degree by the fact that a small part (< 5%) of the material was second eyes in patients with established glaucoma in the other eye. In the control group however this percentage of second eyes was somewhat higher and the observed incidence at manual perimetry still only 0.5%.

Automatic perimetry thus revealed several times more field defects than was observed in the control group while the incidence in the control group was in good agreement with published figures (e.g. Ierkins 1973, Linner 1976, Velje Sørensen et al. 1978).

The high percentage of newly detected field defects when the automatic test was applied most certainly indicates that many *early* glaucomatous defects have been overlooked at the manual routine screening. Sooner or later the manual test would also have spotted the defects which however might be overlooked for several years while still small and difficult to detect. The automatic test often identifies small paracentral scotomas which are easy to miss at traditional perimetry. This result is in accordance with those of Heyl (1976) who found that automatic screening revealed a large number of previously unknown field defects in a material of ocular hypertensives and patients with small to medium sized glaucomatous field defects. These defects were confirmed at conventional static and static profile perimetry.

It must be emphasized however that the results of this study do not warrant conclusions as to the superiority of automatic perimetry as a whole over manual perimetry as a whole. The only comparison that is made is between routine

perimetry as described above and automatic screening on the Competer. In fact the previous study by Heijl (1976) has shown that manual Armaly screening performed under optimized conditions gives results very similar to those of Competer screening. Another study (Heijl et al 1980) shows that the Competer with a threshold measuring test logic works about as well for the detection of defects as an experienced and well trained technician using meticulous combined static profile and kinetic testing with the Tübingen perimeter and both methods miss a few subtle defects. The explanation of the differences observed in the present study is thus not due to a level of perfection in the automatic perimeter which is unattainable at manual perimetry but rather to the fact that routine manual perimetry which is still the major method employed in spotting field loss in suspect glaucoma is unfortunately not very often performed according to those exacting standards which are necessary for a reliable result. One of the many obstacles which prevent the disclosure of early glaucomatous field loss is the non randomized quite predictable stimulus sequence which is normally used at manual perimetry (Heijl & Krakau 1977).

The number of false positives in the first automatic screening of this study is smaller than previously observed. This might at least in part be due to the fairly strict criteria that were used in this study to designate an automatic field as reliable. Only few of these 39 eyes showed field loss later (Fig. 3). This shows that automatic screening repeated once provides a quite good separation between normal and abnormal fields. Most false positive fields fell into a quite distinct pattern with isolated missed points located above the blind spot upwards on the 10° circle and at 20° from fixation.

The fact that the group of field defects confirmed at automatic but not at manual perimetry later proved to contain many cases of early reproducible glaucomatous field loss shows that manual perimetry might easily fail to reveal early glaucomatous defects even if it is performed in a directed fashion – at least if static profile perimetry is not part of the strategy. The same observation has been made before. Some cases denoted as false positives in one study (Heijl 1976) – since they could not be confirmed at manual perimetry – later appeared in exactly the region that had been indicated by the initial automatic screening (Heijl 1977).

Conclusion

The conclusion which emerges is that automatic screening on the Competer is clearly superior to routine manual perimetry as described above for the detection of glaucomatous field defects. We have no reason to believe that this routine manual perimetry is of a lower standard than that performed in the majority of eye departments. It should also be possible to arrive at similar results with other

Most authors agree that the pathogenesis of glaucoma damage of the optic nerve is related to the balance between local blood pressure and IOP (Hawth 1965). The visual evoked response (VER) is sensitive to many types of optic nerve afflictions and indeed several reports have dealt with VER abnormalities in well-established cases of OAG (Ermeris et al 1974; Cappin & Nissim 1975; Band 1978; Huber & Hoyer 1978).

This paper briefly presents the results of an investigation of the VER in a group of persons with long term observation of untreated intraocular hypertension. The effect of a 10-20 mmHg artificial IOP increase is evaluated. All parameters are judged by means of a normal sample ($n = 22$, 1% significance level) and the diagnostic and prognostic perspectives are discussed.

Material and Method

Six men and three women (median age 51 years (42-67)) with a median period of observation of 5 years (2-9) were examined. They had been followed twice a year with examinations including photography of the optic discs and extended field analyses. The IOPs were concentrated in the 22-30 mmHg region and the values of the central corneal thickness did not in any case motivate a correction of the applanatory readings (Ehlers et al 1971). The corrected visual acuity was at least 6/9 and there were no complicating eye or brain diseases.

The VER was recorded from midline occipital and right mastoid electrodes. The central 30° x 22° of the retina were stimulated with a TV generated black and white reversing chequerboard. Large checks (110°) with a 0.9% contrast level were used. Transient VERs were obtained with 3.75 shifts/s and steady state VERs with 1.5 and 30 shifts/s respectively. Transient VERs were measured before and after application of a 1/2 liter spring loaded modulator to the sclera bringing about an IOP increase of 15-20 mmHg. The normal sample resulted in a significant drop in VER amplitude without any change in latency. The steady state responses were evaluated with respect to correct response rate of stimulating frequency. Increase of the IOP had no effect on these VERs in the normal sample.

Results and Discussion

Two persons, a woman aged 59 years and a 12 year-old man, showed no transient VERs with the habitual pressure level but elevation of the IOP resulted in amplitude drops well outside the normal range; the latencies remained normal. The steady state responses were unchanged by pressure loading. The anterior chamber angle was considered normal at most investigations but one obs-

found that a slight mesodermal dysgenesis was possible in the first patient and that discrete signs of bilateral angle contusion could not be excluded in the second patient. Diurnal pressures varied between 22 and 29 mmHg in both cases.

It is tempting to interpretate the VER response in these two cases as an increased pressure susceptibility of the optic nerve. However, such a conclusion obviously demands a considerably longer period of follow up.

Another patient, a man aged 66 years, had been followed for five years with pressure levels of 22-28 mmHg in his only eye, the other having been lost from trauma. The optic nerve head presented a large circular excavation without any vessel displacement or atrophy; the c/d ratio had constantly been 0.8. The usual transient VER showed gross deformation, not allowing any precise latency and amplitude measurements. IOP elevation did not change the pattern appreciably. The steady state responses were normal. Reduction of the IOP to 12 mmHg by means of timolol maleate 0.5% twice a day did not normalize the VER.

This outcome suggests a damaged optic nerve, but whether this is due to a static, possibly congenital defect or to incipient pressure changes can only be determined by further observation.

Another male, 67 years of age, had developed a frank OAG in his right eye since the last control examination without any signs of the same condition as yet from the other. The transient VERs, however, were grossly deformed from both eyes before IOP increase, whereas the steady state responses were normal. With the usual bilaterality of OAG in mind, the present findings point at a high degree of sensitivity of the presently employed VER technique in early detection of glaucomatous lesions of the optic nerve.

In Bartl's investigation (1978) artificial IOP increase and VER were studied in normal and glaucomatous eyes. However, the pressure level had to be above the ophthalmic diastolic pressure before any VER changes were recordable. Possibly, this is explained by the use of smaller checks of lower contrast and a smaller retinal stimulation area. Furthermore, the pattern was shifted with a 5 Hz frequency (10 shifts/s) which means that the response in all probability is a steady state VER, and such were found to be unchanged also in the present investigation.

A more detailed description of the VER technique employed and analyses of normal and pathological samples are under preparation.

Acknowledgment

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PRACTICAL PROBLEMS IN THE USE OF OCUSER[®] PILOCARPINE DELIVERY SYSTEM

BY

PENTTI SIHVOLA and TUOMO PUUSTJÄRVI

The effects of the long acting pilocarpine preparation Ocuser[®] were studied by its application for one week into a total of 50 eyes of 42 earlier untreated glaucoma patients. From the diurnal pressure curves it was found to be equivalent to three daily administrations of 2 or 4% pilocarpine topically. The experiences were as follows: 8 of the preparations had to be removed before the end of the experiment due to insufficient effect and two were removed for other reasons. 19 fell out accidentally, 3 were displaced so as to be visible occasionally under the eyelid but did not fall out, 2 patients succeeded in replacing the preparation after it had fallen out. In the 19 eyes (37%) of all patients still in the trial after the first day the preparations stayed in place without any difficulty for the whole week. All patients experienced minor side-effects but only one of them terminated the treatment due to these side-effects. Serious side-effects did not occur.

Key words: ocuser – pilocarpine therapy, long acting pilocarpine preparation – glaucoma management

One major problem in the treatment of chronic glaucoma is the high pressure values measured in many out patient departments. When the patient is taken into the hospital ward for checking the effectiveness of the medication, it is possible to obtain a very satisfactory diurnal curve with what have had been reported to be an unsuccessful medication programme. One reason for this may be the reluctance to apply eyedrops several times daily to an eye which is completely without subjective symptoms. In addition, old people living alone often have difficulties in applying eyedrops.

For patients with arm or hand injuries and for old and demented patients especially our patients the Ocuser pilocarpine delivery system would seem to be a good alternative. However both Heilman (1975a) and Henning (1976) reported considerable difficulties in the Ocuser treatment one of the greatest was the problem of inserting the Ocuser device properly in the conjunctival cul-de sac.

In this study we have attempted to clarify whether Ocuser P-40[®] is suitable for routine use in certain patient groups.

Material and Methods

The material consisted of 52 eyes from 42 patients with untreated glaucoma randomly chosen from the ophthalmology ward of the University Central Hospital of Kuopio. They consisted of 27 women and 15 men. The age of the women ranged from 44 to 82, average 67 years. The men ranged from 30 to 80 years, with an average of 64.

All the patients were examined with the normal routine glaucoma tests: visual acuity, slit lamp biomicroscopy, gonioscopy, applanation pressure, ophthalmoscopy, Goldmann visual fields and diurnal pressure curves. The diagnosis was based on following criteria: intraocular pressure equal to or greater than 23 mmHg measured by Goldmann applanation and glaucomatous cupping of the optic disc with or without defects in the visual field. After confirming the diagnosis, a further diurnal pressure curve was drawn after administration of three doses of pilocarpine 2%. Unless the results were satisfactory (all readings of pressure under 23) a new curve was constructed after three doses of pilocarpine 4%.

After completion of these tests Ocuser P-40[®] the dimensions of which are 5 × 13 × 0.5 mm was placed in the upper (or sometimes lower) cul-de-sac of the eye. A new diurnal pressure curve was drawn with Ocuser in place. If this curve proved clinically satisfactory, then after confirming that Ocuser was still in place the patient was allowed to return home. After 7 days the patient returned to hospital for interview and a further diurnal curve was made. If Ocuser had fallen out the trial was terminated. In 5 cross-over patients Ocuser was for the first week placed in the right eye and pilocarpine eye-drops in the left eye 3 times daily. For the second week this scheme was reversed.

Results

The 52 eyes included in this trial were diagnosed as follows: glaucoma simplex 31 cases, glaucoma capsulare 24 cases, glaucoma congestivum chronicum 6 cases and glaucoma juvenile one case.

Two or 4% pilocarpine was effective in all cases with the exception of seven cases of glaucoma capsulare and one of glaucoma congestivum chronicum. Ocusert was not effective in these exceptional cases and was removed after the first day.

Two patients from the group voluntarily terminated the trial: one for mental reasons and the other, the 30-year-old glaucoma juvenile patient, experienced so much pain from Ocusert that he wished the trial to be stopped. This patient experienced similar pain after 2% pilocarpine.

In all cases where pilocarpine gave a clinically satisfactory diurnal pressure curve, an equivalent curve was obtained with Ocusert P-40® and was still obtained on the ninth day.

Of the total amount of Ocusert (52) III preparations (37%) had fallen out after one week. 3 had been some partly extended from the eyelids, but the patients had succeeded in replacing them and 2 had fallen on to the hand or slipped onto the cheek, and again the patient had succeeded in reinserting them. Seven units of preparations had distorted forming a figure 8 in the cul-de-sac. Nineteen units (36%) remained in place without difficulty for the whole week. Conjunctivitis or objective symptoms or irritation were not apparent. The average age of the patients whom Ocusert did not remain in place was 70 years. Only one of these had structurally a low cul-de-sac. Other eyelid anomalies or apparent weaknesses in the eyelid were not present.

Only one of the patients made any spontaneous complaint about Ocusert but during the interview all patients complained about irritation during the initial days and 3 had felt a foreign body sensation at the beginning and had noticed some reddening of the eye.

Discussion

Further interest was generated when it was shown that Ocusert caused considerably less accommodation myopia than the equivalent pilocarpine drops (Heilmann 1973a, Leydhecker 1975). In young healthy students four doses of pilocarpine 2% induced up to 10 D myopia, whereas Ocusert caused under half of this amount. In this study the only glaucoma juvenile patient suffered pain explained as ciliary spasm from both pilocarpine 2% and Ocusert.

The equivalent effectiveness of Ocusert and pilocarpine in glaucoma simplex cases has been shown in many reports (Armaly 1973, Heilmann & Sinz 1974, 1975, Heilmann 1975, 1977, Henning 1976, Leydhecker et al. 1975). In this investigation a similar result was also obtained in 5 glaucoma congestivum chronicum and 12 glaucoma capsulare cases in addition to the glaucoma simplex patients.

Heilmann (1975b) reported in his 15-month investigation that in 41% of the patients (total 17) Ocusert remained in place throughout the entire study. The

Table 1
The causes of the failure and difficulties of using Ocuser® and over one week

Difficulties and failures with Ocuser	Number of eyes tested
Fallen out*	19
Fallen out and replaced	2
Displaced	3
Distorted	7
Insufficient effectiveness	8
Patient's reluctance	9
All eyes tested	59
Difficulties and failures all together	41/59
* Absolute disadvantage	19/52 eyes = 37%
Relative disadvantage	99/59 eyes = 49%

result of Hennig's (1976) material of 53 patients tested under five weeks and Ocuser remained in place in 64% of the patients. In this study in 51% of the examined with Ocuser the result was good and compatible (Table 1).

In Hennig's group 28% of the patients complained of irritation in the eyes. In this investigation these symptoms appeared in 4 of the cases but disappeared after 1 or 2 days.

Heilmann (1975a) and Lienert & Busse (1975) noticed that Ocuser could revolve around its longitudinal axis in a figure-of-eight. This lessened its effectiveness and caused irritation in some patients. In this study this was observed in 1 case but seemed to be with minimal subjective symptoms and without perceptible effect on the diurnal curve.

Lienert & Busse (1975) and Leydhecker et al. (1975) noticed that Ocuser in the upper cul-de-sac caused less foreign body sensation than when placed in the lower cul-de-sac. This was also apparent in this study. In the patient who had two shallow lower cul-de-sacs the upper one was sufficiently deep. In some patients Ocuser was observed to move spontaneously from one cul-de-sac to the other. Therefore when Ocuser reached the inner canthus it fell out more easily than when moving past the outer canthus. With the exception of the first two cases all the Ocusers were put under the upper eyelid.

The greatest problem in the use of Ocusert® ocular delivery system seems to be weak permanence in the conjunctival cul-de sac without any present prevention possibilities. The reduction of the tarsal tissue elasticity with age increases the ejection frequency. We have noticed that patients over 70 years have a greater ejection frequency of Ocusert than younger patients. The risk can be minimized if the patients are well instructed by the ward personnel in the use of the Ocusert® system.

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TIMOLOL-MAINTENANCE TREATMENT

BY

B. M. CALISSENDORFF and N. MAREN

Thirty-eight patients with ocular hypertension or glaucoma were treated with topical timolol for 12-30 months (mean 20). A long lasting hypotensive effect of timolol eyedrops was found. However a need to increase therapy during the follow up period was noted especially in the glaucoma group. Eyes with pseudoexfoliations often required concomitant therapy from the beginning. Ocular side effects were relatively few but four cases of punctate keratitis and another two with decreased corneal sensitivity were observed.

Key words: timolol - follow up study - intraocular pressure - glaucoma - ocular hypertension - pseudoexfoliation - side-effects

Timolol maleate, a beta-adrenergic blocking agent, reduces intraocular pressure (IOP) in normal (Katz et al. 1976) and glaucomatous eyes (Zimmerman & Kaufman 1977; Kriegelstein 1978).

The exact mechanism of action is not fully understood, but the main hypotensive effect of timolol is attributed to a reduction of aqueous humour formation (Cawley & Brubaker 1978; Wabloniski et al. 1978; Sonntag et al. 1979).

Timolol is a fairly new substance in the treatment of glaucoma. Thus the question of long term drug efficiency and late side effects cannot yet be fully answered. Maintenance studies with an observation time of one to two years report good tolerance and good drug effectiveness of timolol (Boyer 1978; Daub 1978; Kriegelstein 1979; Lin et al. 1979; Nielsen & Eriksen 1979; Strempel 1979).

This study presents a long term follow up of timolol treated patients with angle glaucoma or ocular hypertension.

Material and Methods

The study included 38 adults (20 males and 18 females) with an untreated intraocular pressure (IOP) > 22 mmHg in each eye. Mean age was 66 years, range 53-77. The patients were observed for 12-30 months (mean 20). Twenty-five subjects were classified as chronic open angle glaucoma fulfilling one or more of the criteria: glaucomatous vision field defect, optic nerve cupping with a cup/disc ratio (CDR) ≥ 1 or documented progress of CDR ≥ 2 . The remaining 13 patients were considered as ocular hypertensions (OH). Exfoliative material was found in 13 eyes: 10 in the glaucoma and 3 in the OH group.

The study is a follow up of patients who previously had participated in a comparative study timolol/pilocarpine (Calissendorff et al. 1980). The admission criteria as well as initial therapy regime is thus based on the protocol from that study.

Timolol treatment was started with 0.25% solution once a day and in cases of insufficient response (IOP > 22 mmHg) increased to 0.5% once or twice a day.

Patients who in the comparative study received pilocarpine were switched to timolol in adequate concentrations.

In the long term study pilocarpine was first choice for additional therapy. Patients who in the comparative study received additional acetazolamide were switched to topical treatment if possible. Only if combined topical therapy did not adequately reduce IOP was acetazolamide added.

Repeated measurements of IOP > 22 mmHg initiated changes in therapy. In some cases an IOP slightly above the stipulated 22 mmHg was accepted if other factors pointed strongly against additional therapy and there was no sign of ocular damage.

The patients were examined at least every three months. Beside ocular examinations (including vision acuity, cotton wisp testing of corneal sensitivity, slit lamp observation, Goldmann's applanation tonometry, funduscopy and visual field test in Goldmann's perimeter) blood pressure and heart rate were registered. Furthermore after one year's treatment with topical timolol diurnal pressure readings were performed.

In those patients receiving acetazolamide the eye with the highest IOP determined the therapy. In some cases topical therapy differed between the eyes and in cases where one eye needed the higher concentration of timolol for the convenience of the patient both eyes have been treated with the same solution. Consequently the presentation of IOP values and therapy changes is based on the eyes with the highest IOPs. In the report of side effects and of cases with exfoliations all eyes are included.

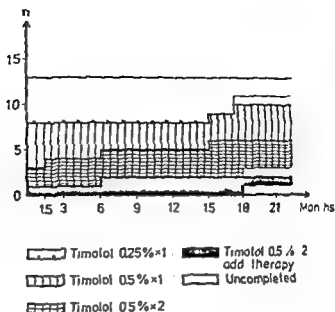


Fig. 1

Distribution of therapy in 19 patients with OHT during 21 months observation. Therapy changes induced by rise in IOI's

Results

The long term effectiveness of timolol maleate was studied for 12-30 months in 5 patients with elevated IOP. During the follow up one patient (age 70 years) died from acute myocardial infarction and 3 dropped out for reasons reported below.

Initially 20 patients could be controlled on topical timolol once a day and another 10 could be controlled with administration twice a day. The number of patients with regulated IOP on topical timolol alone corresponds to 92% of the OHTs and 7% of the glaucomatous eyes. Eight patients in the study had concomitant therapy from the beginning.

Figs. 1 and 2 show the distribution of patients according to treatment and observation time for OHTs and glaucomas respectively. The presentations are restricted to the first 21 months of observation since thereafter the number of examined subjects markedly decreases. Mean without IOI was 29.4 ($SD \pm 11$) mmHg for the OHT and 32.6 ($SD \pm 6$) for the glaucoma group. According to Student's *t* test there is no significant difference between those values ($P > 0.1$). After 12 months 21 patients remained on topical therapy corresponding to 92% of

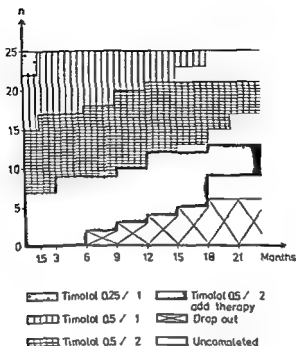


Fig 2

distribution of therapy and dropouts during 21 months observation of 25 patients with glaucoma. Therapy changes induced by rise in IOP

the OHs and 52% of the glaucomas. Thus the main part of subjects needing additional therapy were glaucomas. Compared to the OH group where the therapy remained rather constant the glaucoma group showed a shift towards higher concentration of topical therapy as well as further additional concomitant therapy.

Fig 3 shows the corresponding values for 13 eyes (10 glaucomas, 3 OHs) with contact lens material. Seven of those eyes (54%) needed additional therapy from the start and after twelve months observation only 3 (23%) remained on topical therapy.

Fig 4 presents mean IOP in 8 patients treated with timolol alone in unchanged concentration during at least 21 months observation. The material consisted of 3 patients with glaucoma and 5 with OH; one in each group had pseudoexfoliations. A significant reduction of mean IOP compared to pre-treatment level was seen throughout the observation time and the pressure decrease is not significantly less at the end compared to the beginning of the study (paired *t* test).

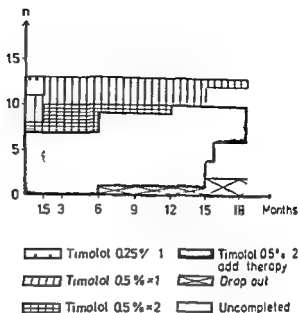


Fig 3

Distribution and changes of therapy in 19 eyes with raised raised IOP and exfoliative material observed during 18 months

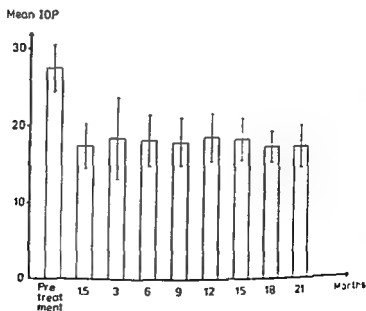


Fig 4

Mean IOP in 8 patients treated with timolol alone in an untreated concentration over 21 months

Table I
Ocular signs observed during study

Decreased visual acuity	1
Visual field defects discovered	6
Visual field defects progressed	16
Punctate keratitis	1
Corneal anaesthesia	
Irritation	
Hyperosmolarity	66
Conjunctivitis	7
Burning itching dry eyes at instillation	

Table I shows ocular signs and symptoms registered during the follow-up period. Some of the findings are probably not related to timolol treatment.

A reduction of visual acuity was observed in 4 patients. Senile macular changes noted pre-treatment were considered to explain the reduction in 1 case, and a slight progress of cataract was observed in 3 patients. Two patients earlier treated with pilocarpine noted increased dependence on their reading glasses.

During the observation time a suspected progress in visual field defect was found in one eye, and new defects were noted in 3 eyes. One of these new defects was observed in a patient whose IOP at all regular observations had been well below 21 mmHg. In spite of maximum therapy 2 patients developed uncontrolled IOP and underwent surgery. One patient not adequately controlled with timolol needed additional therapy and was given pilocarpine. As her IOP was controlled with pilocarpine alone the timolol medication was discontinued.

A slight burning sensation when instilling the timolol drops and sometimes a light feeling of dry eyes was reported by 6 patients. Four patients stated a more pronounced foreign body sensation and were found to have a superficial punctate keratitis. Three of the keratitis cases healed promptly when treated with ointment and continued all the time with the timolol medication. One of the keratitis cases which started after a traumatic erosion needed five weeks before all signs and symptoms were eliminated. During this time timolol eyedrops were excluded. Later treatment was re-administered and the patient has been observed for 11 months without any signs of discomfort or recurrence. Corneal sensitivity was normal in these keratitis cases. Reduced corneal sensitivity was found in 9 patients without any other symptoms. After discontinuing the timolol medication the sensitivity improved within a few days. Eight patients had unspecific conjunctivitis which healed

spontaneously. One patient had an iritis which healed after two weeks treatment with topical atropine and cortisone. Afterwards IOP remained controlled with timolol treatment and it was discovered that this patient had been receiving oral metoprolol for a fortnight. He was therefore excluded from the study.

No significant changes in blood pressure were observed during the follow-up period. In the comparative study a low but significant reduction in pulse rate was observed. However after a long period of timolol therapy pulse was no longer significantly low compared to baseline values (mean pulse rate before treatment 71.3 and after one year 70.3 beats/min). In one of the patients a congenital heart block caused a very slow pulse rate after 6 months observation. Timolol was excluded. The consulting internist considered that no cardiac therapy was necessary but timolol medication was stopped as a precaution. One year later pulse rate still remained low.

Discussion

This study affirms the impression of earlier maintenance studies that timolol has a long lasting hypotensive effect (Boger et al. 1978; Dirsch et al. 1979; Krieglstein 1979; Lin et al. 1979; Nielsen & Enksen 1979; Sirempel 1979). However in many cases in our study a gradual rise in IOP led to an increase in therapy. The development is most pronounced in the glaucoma group where after 11 months observation only 32% remained on their initial therapy compared to 85% in the OH group. Is this a sign of drug tolerance as recorded by Leydecker (1972) and Krieglstein (1978) or a progress in the severity of the glaucoma? No definite answer can be given. The more pronounced increase in therapy in the glaucoma compared to the OH group indicates an aggravation of the illness. The findings in two of our glaucoma patients may also speak against drug tolerance although here a rebound effect as recorded by Krieglstein (1979) cannot be ruled out. One patient without any previous therapy had after 11 months on topical timolol IOPs almost at pre-treatment level. Discontinuation of timolol resulted in a pressure rise clearly above previous pre-treatment level. Re-administration of 0.5% timolol once a day gave a percent age pressure decrease quite comparable to the initial one. The other patient had a capsular glaucoma and was controlled with pilocarpine before she entered the study. Initially her IOPs were regulated with merely timolol but as time went on the IOPs increased. Switching the therapy once again to pilocarpine did not sufficiently reduce mean IOP but after additional timolol adequately controlled IOP was achieved.

Elevated IOP in eyes with pseudoexfoliations is more therapy resistant (Hertv & von 1973, Airaksinen 1979) a finding also reflected in this study. Theoretically reduced aqueous secretion could impair the outflow capacity which might finally endanger a capsular glaucoma. Hitherto reported studies have not confirmed the theory of reduced outflow in long term treatment with timolol (Berer et al. 1978, Dausch 1979, Lin et al. 1979) and special regard was taken to capsular glaucomas in a six month follow up observation by Airaksinen (1979). A reduced aqueous formation might also interfere with normal tear physiology. No certain conclusion can be drawn from our 3 cases of cataract as the disorder is common in this age group. All 3 patients had cataract before timolol was introduced and we have not been able to show any acceleration of the process during the treatment.

The ocular side effects in the present study include two cases of corneal anaesthesia without any subjective symptoms and 4 cases of punctate keratitis with normal corneal sensitivity. van Buskirk reported (1979) corneal anaesthesia from maleate therapy. In his cases as well as ours corneal sensitivity became normal after discontinuation of timolol which indicates a local anaesthetic effect, not a nerve damage. Many β -blockers have membrane stabilizing activity, i.e. an anaesthetic effect, a property which timolol is said to lack (Merck, Sharp & Dohme 1974). However it seems that chronic use of timolol can in susceptible individuals cause corneal anaesthesia.

Superficial punctate keratitis in timolol treated patients have been reported by Mc Mahon et al. (1979) and Nielsen & Enksen (1979). None of the four keratitis in our study had corneal anaesthesia and all have continued with timolol therapy for several months without recurrences. A significant connection between timolol treatment and keratitis is not proved. Hypothetically the corneal epithelium could be influenced by a mechanism similar to that found in rabbits where lacrimal production was reduced by β -blocking agents (Åberg et al. 1979).

Timolol has been shown in the studies cited above as well as in our study to be a good hypotensive drug with rather few side effects. However timolol treated patients should be kept under close observation in order to note any side effect. At present, corneal problems seem to be the major concern if timolol in the long run is to fulfill its promise as a good alternative in the conservative treatment of glaucoma.

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330 TRABECULECTOMIES A LONG TIME STUDY (3-5½ YEARS)

BY

TORD JERNDAL and MATS LUNDSTRÖM

81 eyes out of 330 were followed during 3 to 5½ years after trabeculectomy. 39 eyes were drop-outs due to death and 17 eyes due to inability to participate in the examination program. The mean age at time of surgery was 66 years. A mean pre-operative IOP of 31 mmHg dropped to a mean post-operative level of 18 mmHg. In 57% a single trabeculectomy was considered enough to control the glaucoma. Post-operative medical treatment was considered necessary in 35%. In 87% the pre-operative progress of the field defect was arrested. A reoperation was performed in 8%. The early complications were very few, but in 25% a slowly developing cataract was observed. A cataract extraction was performed in 29 eyes post trabeculectomy with a favourable visual outcome. This study confirms the opinion that trabeculectomy is an atraumatic and efficient surgical procedure and a necessary therapeutic measure when the tolerable combination of antiglaucoma drugs proves insufficient to control the glaucoma.

Key words: glaucoma - glaucoma surgery - goniodysgenesis - microsurgery - trabeculectomy

In 1977 we published a first study of 330 trabeculectomies followed for ½ - 3 years (Jerndal & Lundstrom 1977). Since the long term results after trabeculectomy are not sufficiently documented we decided to re-examine the material 3-5½ years after the operation. With a material of that scope it is our opinion that exhaustive documentation of the potentials of this surgical method may be possible.

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Technique

Most cases were operated under local anaesthesia. Limbus based conjunctival and scleral flaps were performed.

A trabeculectomy specimen was excised including the line of Schwabbe's incision and the scleral spur posteriorly.

A basal iridectomy was made corresponding to the width of the trabeculectomy. For further details see Jerndal & Lundström (1977).

Material

In Table I the number and sex distribution of the original material (330 eyes) and the present one (281 eyes) are specified. 32 eyes were drop-outs due to death and 17 eyes due to inability to participate in the examination program or move to another area.

Table II specifies the glaucoma classification of the operated eyes. The classification is based on meticulous gonioscopy and sometimes modified by the gonioscopic findings during surgery.

We have also closely followed the criteria for glaucoma classification used by the International Symposium on Glaucoma in 1961 and used by Duker *et al.* (1969). The term late congenital glaucoma is defined: glaucoma that is caused by congenital goniodysgenesis and appears after infancy (Kluyters 1960; Fraunfelder 1953).

The high figure for late congenital glaucoma reflects the frequent detection of goniodysgenesis due to the detailed gonioscopic technique.

All patients in the material had progressive field defects before operation and an IOP higher than 21 mmHg except those diagnosed as low tension glaucoma. No cases of ocular hypertension without visual field defects were included.

Table I
Sex distribution

	Males	Females	Total
Number of operated eyes Original study 1977	146	184	330
Number of operated eyes Present study	120	161	281
Drop-out	6	23	29

Table II
Distribution of specified glaucoma diagnosis ($n = 41$)

Late congenital glaucoma	15
Exfoliation glaucoma	10
Simple glaucoma	13
Pigmentary glaucoma	4
Closed angle glaucoma	3
Secondary glaucoma (uveitic)	1
	<i>41</i>

Table III
Mean post-operative reduction of IOP after the trabeculectomy (281 eyes). The post-operative IOP was taken as the mean of the latest two values at ambulatory controls

Mean IOP by appplanation	Period of observation (years)			
	3	4	5	5½
Pre-operative IOP	30	32	31	31
Post-operative IOP	18	16	18	19
Number of eyes	62	93	109	22

Table IV
Number of eyes with post operative IOP ≤ 21 mmHg after the therapy indicated ($n = 244$)

Therapy	Period of observation (years)			
	3	4	5	5½
Single trabeculectomy	37	62	53	9
Trabeculectomy combined with medical treatment	15	20	26	7
Re-operation (trabeculectomy)	3	2	0	2
Re-operation combined with medical treatment	0	2	5	1
	55	86	84	19

Results

All tonometry was performed with the Haag, Streit applanation tonometer and the patient in a sitting position. The pre- and post-operative IOPs are plotted in Table III. A mean pre-operative IOP between 30 and 32 mmHg in the different follow-up groups has dropped to a mean post-operative level of 16.1 mmHg. A slight tendency to a higher figure in the groups followed for 5 years and more is noted.

The figure for the pre-operative IOP was the reading on medical therapy when surgery was decided.

The figure for the post-operative IOP is the mean of the latest two values at ambulatory controls.

In Table IV the eyes with a post-operative IOP ≤ 21 mmHg are divided into four groups to demonstrate the post-operative additional therapeutic measures used.

It is seen that 161 eyes (57.3% $n = 281$) were kept at an IOP ≤ 21 mmHg with a single trabeculectomy. With the various additional measures listed 94 eyes (33.5% $n = 281$) were controlled at or below 21 mmHg.

The remaining 37 eyes are plotted in Table V. Thirteen of these have only had a single trabeculectomy and no post-operative treatment so far, but are candidates for further antiglaucomatous therapy and thus have a very good prognosis for pressure control.

Table V
Number of eyes with post-operative IOP > 21 mmHg
after the therapy indicated ($n = 37$)

Therapy	Length of observation (years)			
	3	4	5	5½
Single trabeculectomy		2	4	2
Trabeculectomy combined with medical treatment	1	6	8	0
Re-operation (trabeculectomy)	1	0	0	0
Re-operation combined with medical treatment	0	1	6	1
	2	1	14	3

Table VI

Number of eyes given post-operative medical treatment. Notice that the figures in the first two columns (1 and 2 years of observation) are taken from the previous study

	Period of observation (years)					
	1	2	3	4	5	5½
Number of eyes with post-operative medical treatment (1%) (29%) (26%) (30%) (44%) (41%)	13	14	16	29	43	9
Number of eyes in each group	86	46	62	93	102	22

A demonstration of the increasing need for post-operative medical treatment is seen in Table VI

Table VII shows the post-operative development of the visual acuity. The most common cause of impaired visual acuity was a slowly advancing cataract.

In Table VIII we have attempted to list those eyes which displayed post-operative progressive field loss attributable to glaucoma. Naturally in the presence of an advancing cataract this was in some cases an almost impossible distinction.

Table VII
Post-operative change of visual acuity (n = 281)

Change of visual acuity		Period of observation (years)			
		3	4	5	5½
> +0.4			3	2	
+0.3	+0.4	2	1	3	-
+0.1	+0.2	4	9	8	-
±0		29	39	96	4
0.1	0.2	11	20	19	7
0.3	0.4	15	19	29	3
0.5	0.6	5	5	10	2
0	0.8	3	3	5	3
> 0.8			3	4	3
		62	53	102	22

Table VIII

Number of eyes showing post-operative progressive field loss due to glaucoma ($n = 231$)

Post-operative loss of field due to glaucoma	Period of observation (years)			
	3 ($n = 67$)	1 ($n = 95$)	5 ($n = 107$)	12 ($n = 62$)
Number of eyes with IOP ≤ 21 mmHg	3	6	8	3
Number of eyes with IOP > 21 mmHg	1	6	2	1
Number of eyes irrespective of IOP (Percentage of each group)	4 (6%)	12 (14%)	17 (17%)	4 (14%)

The early post-operative complications were described in our first study (Jernblad & Lundström 1977). The only late complication in the present study was the development of cataract. In this study we have listed any lens opacities as a complication if they appeared or increased after the trabeculectomy and reduced the visual acuity two lines or more on the visual acuity test chart. According to this definition 70 eyes developed cataract in the post-operative observation period.

In 29 eyes therefore a cataract extraction was performed 1.5 years after the primary trabeculectomy. Various techniques for cataract extraction were used in order to avoid the filtering bleb e.g. extraction through an intracorneal incision or through an inferior corneoscleral incision.

The favourable visual results after these cataract extractions are shown in Table IX.

Table IX

Visual outcome in 29 eyes that were subjected to a cataract extraction after the initial trabeculectomy

Visual acuity	Before trabeculectomy	Before cataract extraction	After cataract extraction
0 - 10	8	0	10
0.1 - 0.3	12		4
CF 1 - 4 m	5	11	3
< CF	4	3	2

Discussion

The introduction of a new surgical method for glaucoma will with necessity encounter a number of objections logical and historical as well as a great amount of scepticism. The scientific approach to meet the objections and cleanse the air is to present the surgical results of trabeculectomy as detailed as possible. This was attempted in our previous paper 330 trabeculectomies (Jerndal & Lundström 1977).

Not only are the details important but also the duration of the post-operative follow up. Thirdly a sufficient number of cases is necessary in order to establish valid conclusions.

With the present study on 281 eyes encompassing 3 5½ years after the original trabeculectomy we feel that valid conclusions are possible. On the other hand it is not meaningful to prolong the follow up of the unselected material further because of the rapidly increasing drop-out frequency. Already at this stage 49 cases have been lost because of death, advanced senility or migration.

The overall post-operative IOP is below 21 mmHg in all groups. A comparison between the 1977 results and the present ones reveals that there is a slight trend for the long term groups to increase in tension. It must be pointed out however that other therapeutic measures than the trabeculectomy were added according to Table IV. It is evident that depending on the etiology and severity of the individual glaucoma a considerable variation of the therapy will be needed.

Table IV demonstrates the post-operative additional therapy resulting in an IOP \leq 21 mmHg. For those eyes not controlled (IOP $>$ 21 mmHg) the therapeutic measures are listed in table V. As can be seen in the latter table 13 eyes out of 37 have so far only had a trabeculectomy and have not yet been put on additional therapy. Most of these eyes have just passed the border of 21 mmHg in IOP and are in no way lost or have a particularly bad prognosis since the therapeutic possibilities are not exhausted.

Eight eyes on the other hand have had the primary trabeculectomy, reoperation and post-operative medical therapy without achieving control of the IOP \leq 21 mmHg. For these eyes the prognosis seems unfavourable unless a new therapeutic breakthrough occurs. β -blocking agents may prove themselves helpful in that category. In the 1977 report we concluded that after 1½ years the need for post-operative medical treatment was stable and approximately 30%. This stable situation also holds for the 4 year group but not for eyes followed for 5 years or more. For these eyes post-operative medical treatment was required in about 40%. It is important to point out that for most eyes with glaucoma the natural history implies a progressively decreasing outflow facility. The same appears to be valid for operated cases of glaucoma but to a lesser extent. Therefore a post-operative

addition of medical treatment will be necessary for a proportion of the cases in our material between 30 and 10%. Similar figures have been reported by others (Loewenthal 1977, Wilson 1977, Pralnic & Savir 1979).

The visual results after trabeculectomy must be viewed against the background of 1) the decay of visual acuity in the elderly, 2) the glaucomatous decay and 3) the decay of visual acuity induced or accelerated by pre-operative anti-glaucoma drugs. It has been demonstrated (Axelsson 1969) that the irreversible cholinesterase inhibitors commonly used as anti-glaucoma drugs have definitely cataract-like properties. This side-effect is particularly unwelcome in glaucoma, since it not only diminishes the vision of the patient but also diminishes the possibilities of the ophthalmologist to examine the optic disc and the visual field.

In an unselected material of 80 medically treated cases of chronic glaucoma subsequently operated (Swegmark, personal communication 1978) the decay in visual acuity during the year prior to the trabeculectomy was almost equal to the post-operative decay one year after trabeculectomy. At the time of surgery visual acuity was worse in 51% of the cases, equal in 34% and better in 15% compared with one year before surgery. Considering this 12-month pre-operative drop in visual acuity, our post-operative results are not alarming (Table VII).

The post-operative drop in visual acuity was in most cases due to the development of cataract, in other cases due to retinal degenerations, vascular accidents, vitreous opacities or other factors.

No report on glaucoma therapy is complete without an analysis of the development of the visual fields. One difficulty, however, is to define what changes are due to glaucomatous damage and what changes are due to retinal degenerations, vascular accidents, cataract, vitreous opacities, old age and poor correction of the refractive state. Therefore a careful evaluation of each patient's efforts must be made. With this in mind, Table VIII may be scrutinized. The aim of the anti-glaucoma therapy is to arrest the glaucomatous process as judged by perimetry. Thus the therapeutic efforts in this material achieved this goal in 24 cases (87%).

The post-operative complications after trabeculectomy are few. To the same extent as in the original study of 1977, no complications have been recorded apart from the cataracts.

It is not known if the surgical intervention per se is a significant cataractogenic incitement unless a direct complication occurs (haemorrhage, prolapsed iris, chamber, etc). Age and genetic pre-disposition as well as a pre-operative drug-induced lens damage are factors that certainly play important roles for the final outcome.

It is most encouraging to find, however, that a cataract extraction after trabeculectomy poses no more surgical problems than an ordinary cataract extraction.

Table X

Comparison of tension results and post-operative therapy after glaucoma surgery. Only the 4 year group of the present study is included

	Greve & Dake (1979)	Jernsdal & Lundstrom
Number of eyes	49	95
Follow up period (years)	4	4
Mean pre-operative IOP (applanation mmHg)	33.0	32.0
Mean post-operative IOP (applanation mmHg)	17.5	16.0
Mean drop of IOP	15.7	16.0
Post-operative medical therapy	9 (18.4%)	29 (30.5%)
Number of eyes re-operated	4 (9.5%)	5 (5.3%)
Total post-operative therapy (medical + reoperation)	13 (30.9%)	34 (35.8%)

Table IX. The psychological problem however may be greater for the patient who may not be so interested in a second operation when the first one according to his opinion made him blind.

Consequently we think that a low risk of cataract development is no contraindication to trabeculectomy.

Comparison of results after different surgical methods is notoriously frustrating because of the multiple discrepancies present. One recent study by Greve & Dake (1979) presenting the four years results after double flap Scheie is sufficiently similar in design to allow a comparison of certain parameters as given in Table X. The double flap Scheie can be described as a thermal trabeculostomy covered with one laminar scleral and one conjunctival flap and as such is very similar to our trabeculectomy technique.

The results are also strikingly similar.

With the present paper we conclude the follow up of this unselected trabeculectomy material. The information from this material is not exhausted however for an appraisal of the survivors after e.g. ten years may add another piece of important information. Our overall experience is that trabeculectomy is an atraumatic and efficient surgical procedure and a necessary therapeutic measure at the therapeutic cross roads i.e. when the tolerable combination of antiglaucoma drugs proves insufficient to control the glaucoma.

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FILTRATION BLEBS IN CORNEOSCLERAL V SUTURED WITH DEXON 7-0 AND DEXON 8-0

BY

JØRGEN KLEENER

In a retrospective investigation the incidence of post-operative subconjunctival filtration with bleb formation were studied in 112 eyes which had undergone cataract surgery with a corneoscleral incision sutured with braided polyglycolic acid sutures (Dexon®). In 61 consecutive cataract operations sutured with Dexon 8-0 there were filtration blebs in 24 (39.3%) eyes and in 51 consecutive cataract operations sutured with Dexon 7-0 there were filtration blebs in 11 (21.6%) eyes. Although there is a difference between the two materials this is not statistically significant with the normal security level $P < 0.05$.

Arterial filtration blebs - corneoscleral suturing - absorbable sutures - cataract surgery

Per 18 1979 Dexon 7-0 was replaced by Dexon 8-0 as the suture material in cataract surgery at the Eye Clinic, Frederiksborg Hospital, Copenhagen. In December 1979 a suspicion of an increased rate of conjunctival filtering blebs arose and the department therefore returned to the use of Dexon 7-0 sutures.

To study the problem more accurately all patients cataract operated with a corneoscleral incision and sutured with Dexon sutures during 1979 were clinically re-examined.

Dexon is the trademark of Davis and Geck, American Cramel Company, Pearl River, New York, 10603.

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Material and Methods

58 eyes were operated in the period 1 1 1979 to 31 7 1979 using Dexon 7 sutures. 118 eyes were operated in the period 1 8 1979 to 31 12 19 3 sutures, Dexon 8 0 sutures. Due to death and major diseases it was only possible to reexamine 51 consecutive eyes sutured with Dexon 7 0 and 61 consecutive eyes sutured with Dexon 8 0. The examination consisted of an evaluation of the corneoscleral wound, an evaluation of possible subconjunctival filtration bleb and an applanation tonometry.

All cases with Dexon 8 0 and subconjunctival filtration bleb were phototaped.

The examination of the Dexon 7-0 material was performed one year after cataract surgery. The examination of the Dexon 8-0 material was performed from 3 to 8 months after cataract surgery.

The microsurgical technique used was the same in all 112 eyes. Lateral based conjunctival flap. Corneoscleral incision. 2 preplaced sutures at 1.30 and 10.30. Opening of the anterior chamber at 12.00 and widening with corneal scissors to approximately 160°. Peripheral iridectomy. Intracapsular extraction of the lens. Tying of the pre placed sutures all as 2 1 1 knots. Finally continuous suturing of the conjunctiva and Tenon's capsule.

All patients received standard topical medication post-operatively. Sixplumex 0.2% twice and ultralanum cum chloramphenicol 1 times daily normally for 6 weeks (5 mg fluorocortone pivalas and 2 mg chloramphenicol in ricinus oleum ad 1 g).

Table I

Age	Number of patients				
	Dexon 7-0		Dexon 8-0		
	Male	Female	Male	Female	
51-60 years	-	-	1	1	
61-70 years	3	3	3	4	13
71-80 years	10	16	9	1	26
81-90 years	6	11	6	14	37
Total	19	32	21	30	92

Table II

Number of sutures	Number of patients		
	+ filtration bleb		- filtration bleb
	Dexon 7 0	Dexon 8 0	Dexon 7 0
4	1	1	
5	6	16	
6	4	6	1
7			
8			1
9			1
unknown		1	

Results

Age and sex (Table I) There was no difference in age and sex between the two examined groups. The corneoscleral wound was closed with Dexon sutures from 4 to 9 interrupted sutures (Table II). In total, 112 eyes were used of which 2 were pre-placed. 23% had 6 sutures. There was no significant correlation between the number of sutures and the appearance of the filtration bleb though the filtration bleb did not appear if more sutures had been placed.

Subconjunctival filtration bleb (Table III) occurred in 24 out of the 61 eyes (39.4%) where Dexon 8 0 was used. When Dexon 7 0 was used 11 of the 51 operated eyes (21.6%) showed a subconjunctival filtration bleb. With the significance levels ($P < 0.05$) there is no significant difference between the

Table III

	Number of patients		
	+ filtration	- filtration	Total
Dexon 8 0	24	37	61
Dexon 7 0	11	40	51
Total	35	77	112

Table IV

Pressure difference post-operative - pre-operative Dexon 8 0 and Dexon 7-0 united

	Numbers of patients	
	+ filtration bleb	- filtration bleb
$\geq + 6$ mmHg	0	7
$+ 1 \rightarrow + 5$ mmHg	2	18
$0 \rightarrow - 4$ mmHg	9	40
$- 5 \rightarrow - 9$ mmHg	16	9
$- 10 \rightarrow - 14$ mmHg	6	1
$\geq - 15$ mmHg	2	0
unknown	0	5

materials. As a whole the frequency of subconjunctival filtration bleb was 31.3% (Dexon 7 0 and Dexon 8 0 included).

The intraocular pressure (Table IV) showed great variation. The difference between post-operative pressure and pre-operative pressure (Table IV) was on average -6 mmHg in those cases where filtration bleb was found, spreading from +1 mmHg to -19 mmHg. In the cases with no filtration bleb the difference between post-operative pressure and pre-operative pressure in average was less than -1 mmHg, spreading from +9 mmHg to -10 mmHg.

The variation of the pressure was so great that even a pronounced post-operative pressure fall cannot be taken as certain evidence for wound leakage with fistulation. It is necessary to look for it.

Discussion

It is usually reported that human limbal wounds in cataract surgery are healed after 10-12 days and firmly closed after 4 weeks (Jaffe 1972, Flaxel & Swan 1969).

Five days after surgery a limbus based conjunctival flap including Tenon's capsule is firmly sealed (Flaxel & Swan 1969). The so-called remodelling starts 8 weeks after surgery and is completed after 2 2/4 years (Jaffe 1972, Flaxel & Swan 1969).

Dexon 7 0 as well as Dexon 8-0 and Vicryl 8 0 are recommended in cataract surgery even for outpatient cataract surgery (Williamson 1976 1977)

Blades (1979) Furgule (1974) Sherman (1979) White & Porks (1974) and Williamson (1974) report almost concurrent good results after the use of Dexon sutures. No marked post-operative tendency for filtration blebs is reported.

Based on suspicion of increased rate of filtration blebs after a period of 6 months of Dexon 8-0 sutures this investigation took place.

Sugar (1975) reports 11 filtration blebs in 156 cataract operations utilizing Dexon 8-0 sutures and 4 in 70 cases utilizing Dexon 7 0. Klemetti (1979) reports filtration blebs in 17 of 108 cataract operations with use of Dexon 7 0 sutures (15.7%). In the present study frequency of filtration blebs is 39.3% for Dexon 8-0 and 21.3% for Dexon 7 0. It is reported (Sugar 1975 Klemetti 1979) that some filtration blebs appear spontaneously. Therefore it must be born in mind that in the present study there is a difference between the Dexon 7 0 and the Dexon 8-0 material in the length of the period from cataract surgery to reexamination.

Katz & Turner (1970) have experimentally shown that the tensile strength of 4-0, 2-0 II and I size of Dexon sutures had decreased 50% at 11 days and about 75% at 15 days. Craig et al. (1975) reported that both 2-0 and 4-0 Dexon sutures underwent a sharp decline in strength already seven days post-operative. Twenty days post-operatives there is no measurable breaking strength left. Blades (1979) reports that Dexon 7 0 sutures disappeared after 43 days and Dexon 8-0 35 days.

Klemetti (1979) concludes: Dexon 7 0 sutures cannot be recommended in cataract surgery in which the long term retention of suture tensile strength is essential for wound healing. This report shows an even more marked tendency for post-operative filtration blebs as those reported by Klemetti (1979). But why this great discrepancy in the different reports about post-operative filtration blebs after Dexon sutures?

The filtration blebs are actually not seen or they appear after discharge of the patient from hospital. A plainly retrospective examination of the medical records had not exposed the real number of conjunctival filtration blebs. Only reexamination of the patients did.

Increased use of post-operative topical medication with steroid could delay the wound healing. Glucocorticoids are well known to have an inhibitory effect on the proliferation of mesenchymal cells and the production of intercellular macromolecules included collagen. With the knowledge from Craig et al. (1975) of the sharp decline in tensile strength of Dexon sutures already after seven days the explanation of a high rate of filtration blebs post-operatively could be the post-operative use of steroids. Sugar (1975) reports use of 0.1% dexamethasone from the 5th day until 3 weeks after surgery. Klemetti (1979) used 0.1% dexa-

methasone 4 times daily from the first day to the 8th day after surgery. The patient in this report received droplets containing 5 mg fluocortinol pivalate and 2 mg chloramphenicol in ricini oleum ad 1 g from the first post-operative day.

Although no exact conclusion can be drawn it seems justified to avoid use of heavy steroids especially in the first weeks after surgery if Dexon sutures are preferred in cataract surgery.

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ON THE FORMATION OF ELSCHNIG'S PEARLS A TISSUE CULTURE STUDY OF REGENERATING RAT LENS EPITHELIUM

BY

PER P. FAGERHOLM

Rat lens epithelial cells were allowed to regenerate on the lens capsule in tissue culture. The major part of the lens fibers had been removed. After a month spherical bodies appeared similar in appearance to Elschnig's pearls. Electron microscopical examination revealed that these spheres consisted of large extracellular vacuoles and phase contrast examination revealed a continuous change in appearance of these spheres. The mechanism behind the tendency to form extracellular vacuoles probably include an increased extrusion of sodium and water from osmotically stressed lens cells.

Ar v r d s rat lens epithelium - after-cataract - Elschnig's pearls - tissue culture - phase contrast microscopy - electron microscopy

Elschnig's pearls, a specific form of after cataract, are sometimes seen on the anterior capsule after lens trauma or extracapsular cataract extraction (Hirschberg 1901, Elschnig 1911). These transparent spheres appear singly or in aggregates varying in individual size up to two mm in diameter. The individual spheres may form and vanish continuously. The tendency to form Elschnig's pearls may be followed in the slit lamp microscope over several years and they may cause severe reduction in visual acuity (Cowan & Fry 1937). Of the several ways of treatment suggested, the surgical approach is now preferred (Gundersen 1966). Histologically the Elschnig's pearl has been described as one giant cell formed by a regenerative attempt of the lens epithelial cells after lens trauma (Cowan & Fry 1937).

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simultaneous increase in tissue mass underneath the capsular folds was observed in four of the specimens; however, little or no regenerative activity could be identified. In all these cases the other specimen in the same flask showed more extensive regenerative signs.

After 4.8 weeks transparent spheres emerged from folds in 9 of the preparations (Fig 2A and B). Generally, there was a continuous change in size and number of these spheres. In three of the specimens a continuous overall increase in size and number of the spheres was noted during the following 4.8 weeks.

Histological and TEM examination

The main cellular regeneration was identified underneath the extremely folded capsule (Fig 3). However, in some of the specimens cellular growth outside the capsular folds was extensive (Figs 4 and 5B). The latter cells were as a rule more like lens fibers in that they were elongated and had very few cell organelles. However, they generally retained their nuclei. Large or swollen cells were sometimes



Fig 3

Epithelial cell regeneration underneath a thickened and wrinkled capsule. Most cells retain their epithelial appearance and no tendency towards extracellular vacuole formation can be seen. Toluidine blue. Bar 100 μ m



Fig 4

regeneration of lens cells mainly outside the capsule disclosed lensfiberlike cells with abundant extracellular vacuoles of different sizes Toluidine blue Bar 100 μ m

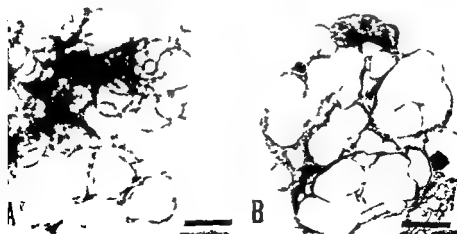


Fig 5

A Phase contrast micrograph of transparent spheres at the surface of a capsule preparation after 10 weeks in tissue culture B Histological section through the transparent spheres revealed large extracellular vacuoles Tolu dine blue Bar 100 μ m

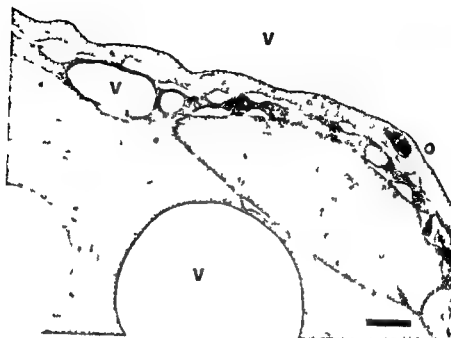


Fig 6

Electron micrograph from the same specimen as in Fig 5. Very few cell organelles are seen in the sometimes extremely flattened cells. Extracellular vacuoles (v) of different sizes were abundant. Bar 1 μ m

identified. In the specimen with this latter type of cellular regeneration extracellular vacuoles were abundant. The size of these vacuoles could be measured up to 0.1 mm in diameter (Fig 5A). The lensfiber like cells in between these very large vacuoles were extremely thin down to 0.3 μ m (Fig 6).

Discussion

In the slit lamp microscope and on histological examination Elschnig's pearls have been regarded as giant cells (Cowan & Fry 1937). The technical problems inherent in the method of surgical removal (Gundersen 1966) probably disrupt these delicate structures and thus explain the scarcity of morphological studies.

The regeneration of human and rabbit lens epithelium in tissue culture has previously been demonstrated using flat mount preparations (Fagerholm & Philip-

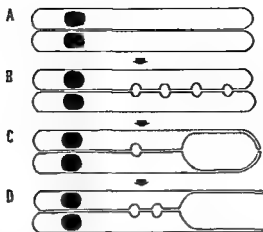


Fig 7

Schematic drawing showing a possible mechanism behind the formation of transparent spheres. The lens fiber like cells (A) respond to osmotic stress influenced by the untoward ionic composition in the aqueous humor by forming extracellular vacuoles (B). These vacuoles coalesce to form giant vacuoles (C) that eventually rupture (D) and the process is then restarted.

son 1977). The present study was focused on the structures that in the phase contrast microscope are very similar to Elschnig's pearls. These structures were not observed in the flat mount preparations of human and rabbit lens capsule, probably due to the shorter observation period, less than of six weeks (Fagerholm & Philipson 1977). The tissue culture procedure with a free floating capsule allowed atraumatic preparation for the electron microscopical examination.

The transparent spheres were shown to be extracellular vacuoles. The vacuoles were surrounded by often very thin cells resembling lens fibers. The change in appearance suggests a continuous formation of extracellular fluid by the lens fiber like cells. The rat lens epithelium regenerated, tending to differentiate to lens fibers. The cells elongated and lost most cell organelles but retained their nuclei. Normally, the lens fiber differentiation takes place inside the lens capsule. The ionic milieu here is compatible to the restricted capacity of mature lens fibers to resist ionic changes (Fagerholm 1979). However, differentiation in the aqueous will probably reach a point where the lens cells have difficulty in withstanding the ionic load of the aqueous. Lens fibers are probably reacting to osmotic stress by increasing the transport of sodium and thereby water into the extracellular space. Extracellular vacuoles will then be formed (Sakuragawa et al 1975). This mechanism (Fig 7) and the appearance and dynamics of the spheres are very similar to the behaviour and appearance of Elschnig's pearls (Cowan & Fry 1937).

Elschnig's pearls as seen in the slit lamp microscope may consist of several types of formations including giant extracellular vacuoles, Morgagnian spheres and perhaps enlarged lens fibers. Several types of cellular response constitute the different forms of after cataract. In the absence of inflammation the end result seems to be dependent on the degree of mitotic activity and the degree of cellular differentiation. The latter probably determines such factors as production of new capsule material as well as the degree of extracapsular vacuole formation.

Further studies are required of which factors determine cellular properties in order to find a non surgical treatment of after-cataract.

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CENTRAL CORNEAL THICKNESS AND INTRAOCULAR TENSION IN PATIENTS WITH ACROMEGALY

BY

THORKILD BRAMSEN ANNE KLAUBER and PER BJERRE¹

In 27 patients with pituitary adenomas the central corneal thickness and the intraocular tension were measured. Thirteen of the patients were suffering from acromegaly and in this group the central corneal thickness was $0.561 \text{ mm} \pm 0.030$ ($\bar{x} \pm \text{SD}$). In the 14 patients with pituitary adenomas but no acromegaly the central corneal thickness was $0.506 \text{ mm} \pm 0.030$ ($\bar{x} \pm \text{SD}$). This difference is statistically significant ($0.01 > P > 0.001$).

In the 13 patients with acromegaly the intraocular tension measured by applanation was $16.9 \text{ mmHg} \pm 2.3$ ($\bar{x} \pm \text{SD}$) and in the control group $14.7 \text{ mmHg} \pm 2.4$ ($\bar{x} \pm \text{SD}$). This difference is statistically significant ($0.05 > P > 0.001$). When the applanation reading is corrected for the difference in the central corneal thickness the patients suffering from acromegaly have an intraocular tension of 14.1 mmHg which is of the same magnitude as the tension in the patients without acromegaly.

Key words: acromegaly - central corneal thickness - pituitary adenoma - intraocular tension

In adults growth hormone producing pituitary adenomas often result in acromegaly characterized by growth of peripheral parts of the body i.e. bones as well as soft tissues. Important to the general appearance is the thickening of the facial soft parts.

Decrease of the acromegalic changes of the soft tissues is an important indicator of successful treatment. No methods exist for measuring this. Growth hormone analyses are of limited value because the correlation between plasma growth hormone level and clinical activity of the disease is poor (Lundholm 1979). If the cornea takes part in the thickening of the soft tissues, measurement of the central corneal thickness (CCT) which can be performed with great accuracy, would possibly be of importance in the diagnosis and the control of the acromegalic patient. Ehlers & Bramsen (1978) found in 45 conscripts a CCT of $0.510 \text{ mm} \pm 0.027$ (mean \pm sd).

Increased intraocular tension has been described with a high frequency in patients with acromegaly (Aren & Skårse 1955; Howard & English 1965). If the CCT increases with acromegaly, it would be assumed that the applanatory values will increase and thus falsely indicate increased intraocular tension (Ehlers et al 1975).

The purpose of this investigation has been to measure the CCT and the intraocular tension in patients with pituitary adenomas with and without acromegaly.

Material and Methods

The material consists of 27 patients with pituitary adenomas verified by X-ray examination or operation. CCT was measured with a modified Haag Streit pachometer as described by Ehlers & Sperling (1977).

The coefficient of variation of the CCT measurement is for a trained examiner about 1% (Olsen et al 1980). The intraocular tension was measured by applanation tonometer as described by Goldmann. Measurements were performed by the two ophthalmological authors and the mean value of the two eyes was used in the calculations. The examining ophthalmologists were unaware of the case records and the diagnosis. Obviously, acromegaly could often be identified at a glance. The participating neurosurgeon was ignorant of the CCT until the conclusion of the study.

Included were 13 patients with acromegaly, seven females and six males. The average age was 46.7 years ranging from 23 to 68 years. The duration of the disease varied from 1.5 to 17 years.

In the group of pituitary adenomas without acromegaly were included 14 patients, eight females and six males. The average age was 43.1 years ranging from 23 to 63 years. The duration of the disease was from 0.5 to 17 years.

Table I

CCT applanation readings and corrugated applanation readings = intraocular tension in patients with pituitary adenoma with and without acromegaly

	Patients with pituitary adenoma	
	with acromegaly (N = 13)	without acromegaly (N = 14)
CCT mm \pm SD	0.561 \pm 0.035	0.526 \pm 0.030
Applanation readings mmHg \pm SD	16.9 \pm 2.3	14.7 \pm 2.4
Corrugated applanation readings Intraocular tension mmHg \pm SD	14.1 \pm 2.3	14.2 \pm 2.4

Results

The result of the measurements of the CCT and the intraocular tension appear from Table I

The difference in the CCT between the two groups is significant $0.01 > P > 0.001$

The difference in the direct applanation readings between the two groups is significant $0.05 > P > 0.02$

No difference in corrugated applanation readings is found between the two groups

Discussion

The investigation has shown that in patients with acromegaly the CCT is increased compared to patients with pituitary adenomas without acromegaly suggesting a hypertrophic effect of growth hormone on the corneal tissue

The present investigation does not show which layers of the cornea are responsible for the increased CCT. At slitlamp examination and during the pachometer measurements the cornea appeared normal especially without any signs of oedema. Within both groups a considerable variation in the CCT was noted. In the acromegaly group this might be explained by different levels of endocrine activity. An investigation on this has been started. In the control group there are two

patients who are suspected of having acromegaly and where only a period of observation can decide the final diagnosis.

The investigation also demonstrated increased applanation values in patients with acromegaly compared to patients with pituitary adenomas without acromegaly.

As mentioned in the introduction, a connection has already been noticed between increased applanation readings and pituitary adenomas. Especially during the 1960s this led to discussions (Cottrifredsen 1968). Naturally patients may occur with both glaucoma simplex and acromegaly as independent diseases. The increased applanation readings in the acromegalic patients of this investigation are due to a misinterpretation of the measured tension values, as it has already been shown (Ehlers et al. 1977) that an increased CCT leads to higher applanatory values. If the applanation readings in this group corrected for the increased CCT, the acromegaly patients have a true intraocular tension of $16.9 \text{ mmHg} - 2.8 \text{ mmHg} = 14.1 \text{ mmHg}$, which is of the same normal magnitude as in the group without acromegaly. The corresponding figures for patients with pituitary adenomas but no acromegaly were $11.7 \text{ mmHg} - 0.5 \text{ mmHg} = 11.2 \text{ mmHg}$.

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ON THE OPTICAL MEASUREMENT OF CORNEAL THICKNESS

II The Measuring Conditions and Sources of Error

BY

THOMAS OLSEN CARSTEN BO NIELSEN and NIELS EHLERS

The optical measurement of corneal thickness based on oblique viewing of the optical section of the cornea is complicated by the finite width of the incident slit beam. In this report the theoretical and practical aspects of the effect of the slit width on the thickness reading are analysed. In practice it was not possible to make slit width independent thickness readings which were reproducible from one observer to another. In addition the observed slit width error was found to vary from one patient to another. The lack of a reproducible estimate of the corneal thickness is attributed to difficulties associated with an exact definition of the edges of the visible bands of the optical section which are determined by biological properties of the cornea as well as perceptive properties of the observer.

Although inter-observer errors up to 0.02 mm were found the intra-observer error amounted to only 0.005-0.006 mm (SD) between consecutive readings. Presumably this high intra-observer reproducibility is the result of the auxiliary pin light used. Changes in corneal thickness measured by the same observer can therefore be determined with great accuracy.

Key words: corneal thickness - pachometer - reproducibility

The optical measurement of corneal thickness (pachometry) is becoming increasingly employed in ophthalmological practice. One of the most widely used methods is based on the principle described by Jaeger (1952) involving oblique viewing of an optical section of the cornea produced by an incident slit light normal to the corneal surface. One advantage of this method is that the theoretical

performance and errors of the measuring system can be calculated in a straightforward manner (Olsen et al 1980). However, despite the theoretical simplicity, the "normal" values reported by different investigators still differ considerably even with the same commercially available equipment. It therefore seems that the measuring conditions need some considerations. We report herein an analysis of some of the practical errors concerned with the optical measurement of corneal thickness.

Methods

A Haag Streif 900 slit lamp with pachometer attachment was used throughout the experiments. To secure perpendicular direction of the slit beam on the cornea a pin light attachment (Ehlers & Sperling 1977) was mounted on the pachometer attachment according to the principle of Mishima & Hedbys (1968). The pin light attachment can be purchased from VOCO, Copenhagen, Denmark. The luminance of the slit light was always kept at a maximum (using over voltage 7.5 V with lamp 6 V, 4.5 V). We were not able to determine the exact luminance of the slit light. However, when the focused slit beam of 0.03×18 mm was entered into a lux meter (window 14×28 mm, normally used for the Goldman perimeter) the reading was about 180 lux. Room light was dim (less than 20 lux). The examiners (N.E., T.O., C.B.N.) had normal visual acuity and were adapted to room light at least 10 min before the thickness readings were started. They were all trained thickness examiners (several months to several years of daily experience). All series of thickness measurements were performed in a blind fashion. That is, the alignment of the two half images of the beamsplitter was done by the examiner, while the scale of the pachometer was read by another observer without telling the examiner. Each reading was taken as the closest 0.01 mm position on the scale reading of the pachometer. The readings were used directly, no corrections being made for corneal curvature or non-linearity of the pachometer. Between each reading the pachometer was put out of adjustment.

The width of the focused slit light was measured by counting the number of 0.01 mm divisions of an object micrometer (Leitz) perpendicularly illuminated by the slit, while the slit image was viewed through the pachometer eyepiece. The reading was reproducible to 0.003 mm.

For multiple comparisons of means, analysis of variance was applied (Sokal & Rohlf 1969).

Results

Theoretical considerations

The optical section of the cornea made by the slit light is a three-dimensional structure. The antero-posterior dimension is made by the cornea itself, the width is made by the slit width. When the corneal section is produced by very narrow and almost parallel beams of light and viewed at an angle of 40° (= angle used in the Haag Streif pachometer), it is usually composed of a broad anterior bright band and a

somewhat optically empty central zone and a narrow posterior bright band. The anterior band results from scattering of the light in the epithelial layer. The width of the anterior band (seen obliquely) is therefore determined for its most part by the thickness of the epithelium and for a smaller part by the width of the slit light. The small width of the posterior band makes it appear as determined solely by the slit width. Because the optical principle of the pachometer assumes no slit width, the correct alignment of the two half images formed by the beamsplitter of the pachometer is therefore presumably not an end-to-end alignment of the optical section, but rather a side-to-side alignment of the (left) slit edge (Fig. 1 alignment A and B respectively).

An assumption of the latter alignment is that the width of the posterior band corresponds to the width of the focused slit light.

Although the exact anatomical basis for the posterior band of the optical section is unknown, it seems evident that alignment A in Fig. 1 would result in an over-

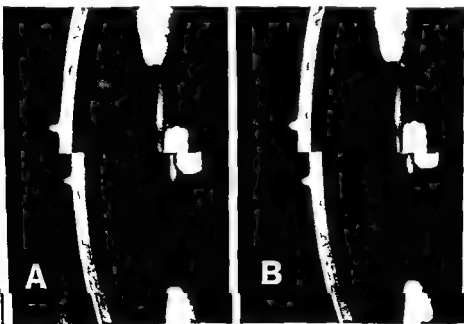


Fig. 1

Optical section of the cornea viewed through the beamsplitter of the pachometer. Two pin lights are reflected at the anterior limit of the section. In A an end-to-end alignment of the optical section is shown. If the narrow posterior band is assumed to represent the slit width, a presumable slit width independent alignment is shown in B.

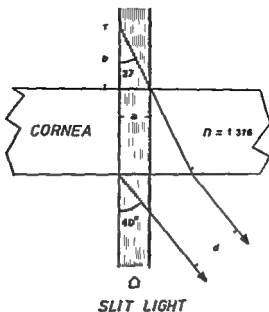


Fig. 2

When the optical section of the cornea is produced by a slit beam of measurable width (a) the slit width is thought by the pachometer to represent an additional thickness (b)

estimation of the corneal thickness. Assuming that the width of the posterior band corresponds to the width of the focused slit light, the expected magnitude of this overestimation can be calculated as follows (Fig. 2).

The angle of observation with the pachometer is 40°. But due to refraction at the corneal surface the slit width (a, Fig. 2) is seen at an angle which approximates 37° (from $\sin i / \sin r = n$ assuming a plane cornea). Through the eyepiece of the pachometer the slit width is seen as an additional part (b, Fig. 2) of the optical section, the thickness of which is the slit width multiplied by $\tan^{-1} (2^n) = 1.3$. (Again assuming no curvature of the cornea). If the corneal curvature is included in the considerations, the true angle at which the slit width is observed becomes smaller, and the corresponding thickness of the slit width becomes greater.

Although the Haag Streiff model 900 slit lamp is provided with a scale reading for the slit width adjustment, it is not calibrated for a direct metric reading of the focused slit width. In Fig. 3 is shown the actual slit width (lower unbroken line) as a function of the scale reading on the slit lamp (valid for the slit lamp used for the present study). We have found this conversion function to vary slightly from one Haag Streiff 900 slit lamp to another. Also the slit luminance was found to vary. The lower part of the curve was seen to stabilize at about 30 μm , corresponding to a reading of 8 or less, below which the slit was only faintly visible. Dotted line (closed

MM SLIT WIDTH/ERROR

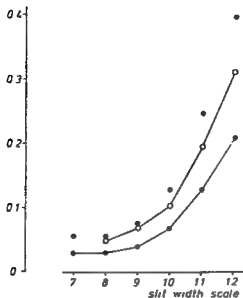


Fig 3

Closed circles unbroken line width in mm of focused slit light as a function of the arbitrary slit width scale on the Haag Streit slit lamp. Closed circles broken line theoretical slit width error on the pachometer reading. Open circles unbroken line observed slit width error calculated as the difference between the A and B readings shown in Fig 4.

circles) in Fig 3 indicates the expected error with alignment A (actual slit width \times 9) on the corneal thickness estimation.

Thickness measurements

In order to test the validity of the above mentioned considerations the corneal thickness of a normal 27 year-old subject was measured with alignment A and B using a number of different slit width settings (Fig 4). As expected the corneal thickness readings with alignment A were greatly influenced by increasing slit width. The readings with alignment B were however also slit width dependent but in the opposite direction (one way analysis of variance showed $P < 0.001$). The difference between the two readings i.e. the apparent slit width error has been included in Fig 3 (open circles). It is seen to be lower than the expected error.

The visible width of the posterior bright band of the optical section did thus not correspond to the directly measured width of the slit.

MM PACHOMETER READING

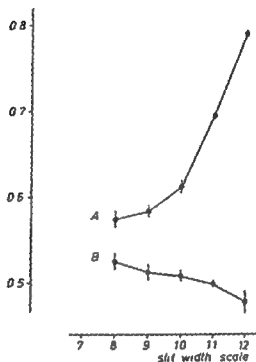


Fig. 4

Pachometer reading as a function of slit width using two methods of alignment A and B. Each point is the mean of six determinations. Bar = SD.

In order to study the reproducibility of the corneal thickness, whether the apparent slit width in the posterior corneal varies, six corneas (from six patients with senile cataracts) were measured with the pachometer using alternating slit widths of 30 and 45 μ m. The expected difference in thickness was 7.7 μ m (30 μ m \times 1.9). From Table I it is seen that this was the case in all of the cases.

Analysis of variance (Table II) showed the mean thickness to differ among the three examiners for alignment A. The largest observed difference in means was 0.022 mm with alignment B. Although the inter-observer difference was well as in alignment B, this was appreciable (standard deviation) was only 0.0021 mm and 0.006 mm.

Table I

Mean value of central corneal thickness readings ($n = 6$) and their standard deviations (in parenthesis) obtained using two methods (A and B) of alignment. The difference between the two readings is the apparent slit width error. Actual slit width is 0.030 mm which theoretically should induce an error of 0.057 mm (see Fig. 2).

Examinator	\ E			T O			C B \		
	A	B	A B	A	B	A B	A	B	A B
Patient \ o									
1	0.568 (0.006)	0.543 (0.007)	0.025 (0.006)	0.569 (0.004)	0.542 (0.005)	0.028 (0.005)	0.584 (0.005)	0.553 (0.010)	0.032 (0.011)
2	0.509 (0.005)	0.477 (0.005)	0.033 (0.004)	0.513 (0.004)	0.468 (0.005)	0.045 (0.006)	0.531 (0.004)	0.481 (0.012)	0.050 (0.012)
3	0.537 (0.007)	0.512 (0.008)	0.025 (0.010)	0.545 (0.003)	0.506 (0.005)	0.039 (0.006)	0.556 (0.007)	0.518 (0.007)	0.038 (0.012)
4	0.548 (0.005)	0.516 (0.005)	0.032 (0.008)	0.538 (0.003)	0.508 (0.004)	0.031 (0.004)	0.543 (0.006)	0.507 (0.006)	0.036 (0.003)
5	0.538 (0.005)	0.522 (0.005)	0.016 (0.007)	0.544 (0.004)	0.518 (0.004)	0.026 (0.004)	0.547 (0.006)	0.515 (0.005)	0.032 (0.007)
6	0.563 (0.006)	0.536 (0.006)	0.028 (0.004)	0.574 (0.004)	0.555 (0.005)	0.019 (0.009)	0.583 (0.005)	0.551 (0.006)	0.033 (0.005)

respectively. The intra-observer A error was significantly ($P < 0.05$) less than the B error. The intra-observer mean difference between reading A and B, that is the apparent slit width error, varied from 0.016 to 0.050 mm and was found to differ from one examiner to another but also from one patient to another. In all three instances, alignment A and B and their difference, a significant interaction was found between the patient and the examiner.

Table II

Two-way analysis of variance of data summarized in Table I

Source of variance	df	F Value		
		A	B	A B
Between rows (patients)	5	not tested	not tested	14.3
Between columns (examinators)	2	51.5 *	4.0	15.6
Interaction	10	5.5	5.2	2.9

$P < 0.05$ $P < 0.001$

Discussion

The intra-observer readings in the present study showed a high degree of reproducibility. For example, a standard deviation of 0.003 mm (about 1%) as found for alignment A (Fig. 1) means that a given single reading was accurate to ± 0.010 mm (95% limit). Presumably this small intraobserver error is the result of the auxiliary pin lights used which secure a perpendicular corneal illumination and a fixed observation angle. A similar small error was reported by Donaldson (1966) who was the first to apply auxiliary pin lights in order to increase the stability of the measuring situation.

However, an inter-observer reproducible estimate of the corneal thickness was not possible with the present methods of measurement. The reason for this may be indicated in the slit width error analyses. Based on edge alignments of the posterior band of the optical section the actual error produced by the posterior band on the thickness reading was found to be smaller than expected from direct measurements of the slit width. This indicated that what is seen as the posterior band of the optical section is dependent on biological properties of the cornea in addition to physical properties of the slit light. The biological nature of the apparent slit width was also illustrated by the different errors produced from one patient to another. Furthermore, the slit width error differed from one observer to another, indicating that the recognition of the posterior band is subject to perceptive variability.

After

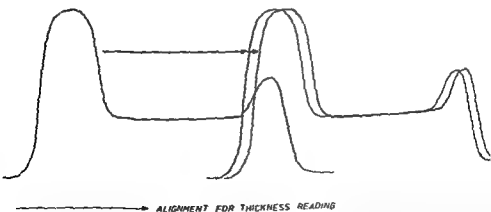


Fig. 5

Model of optical conditions for thickness alignment (Scatter profile modified from McCally & Farrell (1976)). Displacement of the scatter profile in figure corresponds to the horizontal displacement of the two half images formed by the beam splitter. Because the slit edge cannot be defined exactly several alignment possibilities exist.

The light scattered by the cornea when sectioned by a narrow slit beam has been studied by McCally & Farrel (1976). Their results clearly illustrate the alignment conditions and the difficulties associated with a reproducible estimate of the corneal thickness (Fig. 5). What is interpreted as bands in the optical section is not perfect images of the slit but rather a sinusoidal distribution of light with no sharply defined edges. When alignment of edges has to be performed the observer must decide that at a certain level of scatter he will choose this as the edge. From this it seems evident that the alignment for thickness reading has a subjective component. For alignment A an end-to-end alignment of the image of the optical section is performed. If a very small change in light scatter from the baseline is considered as an edge the alignment of corresponding points on both sides of the optical section would result in a high thickness reading as compared to an A alignment which defines a larger change in light scatter as the edge. In alignment B a side-to-side alignment of the edges of the anterior and posterior bands of the optical section is attempted. The two sides are however not superimposable and again the thickness reading may vary according to which points are chosen as corresponding points on the scatter profile (exemplified in Fig. 5).

Not only alignment A but also alignment B was found to be slit width dependent. The reason for the latter observation is not clearly understood. In continuation with the considerations above it means that the corresponding points on the scatter profile are moving apart as the slit width decreases. The posterior band differs from the anterior band both in intensity and in edge resolution. It may be speculated that as the slit width decreases the edges of the posterior band may flatten somewhat so that if the measuring points are defined at a certain level of light scatter they will move slightly apart.

The lack of a reproducible estimate of the corneal thickness thus lies in the difficulties in exact definition of the edges of the bright bands of the optical section. For the same reason it is not possible to make exact corrections for the slit width error.

In order to improve the inter-observer error somewhat we suggest that the actual slit width is always measured and stated in clinical studies on the thickness of the cornea in addition to the method of alignment and slit lamp characteristics. Even then the present study shows that the normal values may differ on the second decimal point. Hence it would be very desirable if reproducible measuring points could be defined for the obliquely viewed optical section. The present angle of observation and the optical resolution of currently available slit lamps does not allow the anterior broad band of the optical section to be divided into two minor bands produced by the slit beam as it passes through the anterior and posterior layers of the epithelium. The study by McCally & Farrell (1976) suggests that this two-band resolution of the anterior band may be possible for a very narrow slit

ATTER

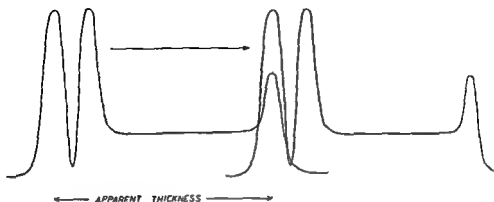


Fig 6

If the broad anterior band of the optical section could be resolved into two narrow bands, it might be possible to define a reproducible alignment method for thickness reading

and/or a large observation angle. If this could be accomplished for the pachometer setting it would be possible to define the center of the anteriormost band and the center of the posterior band as corresponding measuring points (Fig 6). In this situation the thickness alignment would be unbiased by the slit width as well as observer independent. Unfortunately this is not possible with present standard systems.

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THE EFFECT OF CARBONIC ANHYDRASE INHIBITION ON CENTRAL CORNEAL THICKNESS AFTER CATARACT EXTRACTION

BY

CARSTEN BO NIELSEN

The influence of acetazolamide (a carbonic anhydrase inhibitor) on central thickness of the human cornea *in vivo* was studied. Corneal thickness as measured after cataract surgery was significantly increased by acetazolamide if the specular microscopy revealed central corneal guttae pre-operatively. When no guttae were seen by specular microscopy, no effect could be demonstrated.

Key words: corneal thickness - endothelium - carbonic anhydrase - acetazolamide

Normal function of the limiting layers of the cornea is crucial for maintaining stromal hydration. The endothelium is believed to function on basis of a pump-leak theory (Mishima & Kudo 1967, Trenberth & Mishima 1968, Maurice 1972). The active component in rabbit endothelium has been characterized by dependence on bicarbonate (Hodson 1971, 1974, Dikstein & Maurice 1972) and carbonic anhydrase (Fischbarg & Lim 1974, Hodson & Miller 1976, Hull et al 1977) for conversion of exogenous CO to HCO₃. Silverman (1973) found carbonic anhydrase in the endothelium in the same order of magnitude as in most secretory tissues.

The previous studies were performed on rabbit corneas *in vitro*. The purpose of the present study was to reveal the possible significance of carbonic anhydrase activity in the human cornea *in vivo*.

As no effect on normal corneal thickness was found when a carbonic anhydrase

inhibitor was administered (unpublished data) patients to be cataract extracted were chosen. The post-operative condition comprising a hyperhydrated state would seem to favour the possibility of showing a decrease in the deswelling capacity of the endothelium.

Patients with cornea guttata were considered a separate group. These patients are seen to have a different CCT response to cataract surgery (Olsen 1980) and a higher incidence of postoperative corneal oedema (Jaffe 1976) probably as a sign of endothelial malfunction.

Material and Method

Material The material comprised 50 consecutive patients with senile cataract. Patients with other past or present eye disease or intraocular pressure > 21 mmHg were omitted. The first 20 patients received acetazolamide and the next 30 served as control. In the drug group 3 of the 20 were excluded (2 with surgical complications, 2 with striae oedema leading to uncertain CCT measurements and 1 due to drug intolerance). In the control group 3 were excluded (1 with surgical complications, 1 with striae oedema and 1 with post-operative hyphaema).

All operations were performed with the same technique (corneal incision, cryoextraction running 10–0 nylon suture) by 6 surgeons. No significant difference in corneal first post-operative-day thickness of the patients operated by the different surgeons could be demonstrated.

Method The endothelium of all eyes to be operated on were examined by non-contact specular microscopy as described by Olsen (1979). 3–4 exposures were taken of each cornea paying attention to the possible presence of central corneal guttae. Guttata were defined as round dark areas comprising defects in the endothelial reflex larger than two endothelial cells and changing in size with the focusing. If more than one defect defined as above were present on either exposure the cornea was classified as guttata.

Further the central corneal thickness (CCT) and intraocular pressure (IOP) were measured. The CCT measurements (mean of 3 readings to the nearest 5 μ m) were performed with a modified Haag Streit pachometer (Ehlers & Sperling 1977) using the technique (alignment 1) described by Olsen et al (1980) with a coefficient of variation ~ 0.01 . IOP was measured with applanation tonometry.

Experiments The drug group included a total of 20 patients. Based on the pre-operative specular microscopy 11 patients were classified as normal (age 67.1 ± 3.8 mean \pm SEM) and 12 as guttata (75.1 ± 2.6). The 27 patients to serve as control comprised 14 normal (age 69.1 ± 1.6) and 13 guttata (73.0 ± 1.62).

The patients to be medicated were given acetazolamide (Diamox® Duplex, Lederle) 500 mg $\times 3$ for 3 days starting on the first post-operative day in the evening ending on the fourth day in the morning.

The CCT was measured at the same time of the day pre-operatively and daily from the first to the sixth post-operative day. IOP was measured pre-operatively and daily from the second to the sixth post-operative day.

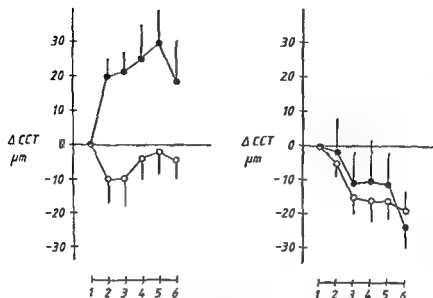


Fig 1

CCT (mean \pm SEM) relative to the first post-operative-day value for the operated eye
 Left guttata Right normal Closed circles after acetazolamide Open circles control

Results

The aim was to test the ability of the cornea to deswell from a given thickness during simultaneous acetazolamide medication. It was decided to let the individual CCT as recorded on the first post-operative day be the zero value. Doing this a contamination based on interpersonal variation in CCT as measured in absolute numbers was avoided. The mean increase in CCT on the 1 post-operative day relative to the pre-operative value was not significantly different from group to group (normal/drug $63.8 \pm 11.1 \mu\text{m}$ mean \pm SEM normal/control $70 \pm 6.1 \mu\text{m}$ guttata/drug $66.6 \pm 9.3 \mu\text{m}$ and guttata/control $74.6 \pm 12.5 \mu\text{m}$).

The corneal deswelling in the 4 groups are shown in Fig 1. Acetazolamide did not affect the deswelling rate of the normal corneas whereas guttata corneas actually swelled. On all days were the CCT values recorded in this group greater than the value on day 1. Considering the group control/guttata a secondary rise in CCT is found on day 4 and 5. The mean CCT in the guttata/drug group are different from control by approximately $30 \mu\text{m}$ (day 1 and 2 $P < 0.01$ day 3 and 4 $P < 0.05$ *t* test).

The IOP on each day was not found to be significantly different between any two groups (Fig 2).

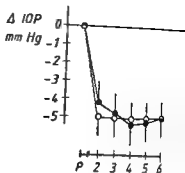
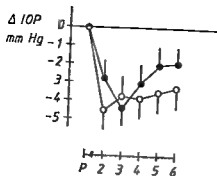


Fig 2

IOP (mean \pm SEM) relative to the pre operative (P) value for the operated eye Left guttata Right normal Closed circles after acetazolamide Open circles control

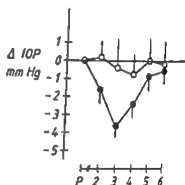
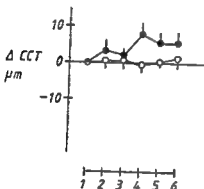


Fig 3

CCT (mean \pm SEM) relative to the first post operative day value and IOP (mean \pm SEM) relative to the pre operative (P) value Both recordings are for the guttata fellow non operated eye Closed circles after acetazolamide Open circles control

Fig 3 shows CCT and IOP for the non-operated fellow eye in the guttata groups CCT in the drug group is greater than control on day 4 ($P < 0.02$ *t* test) Between the normal groups no such significant difference was found neither considering CCT nor IOP (not shown)

Discussion

The aqueous concentration of the lipidsoluble acetazolamide in steady state is assumed to be equal to the free active fraction in plasma (Davson 1950) and so the effective concentration would be the same The dose was 7–10 mg/kg giving an

aqueous concentration of $0.5 - 1.0 \times 10^{-5}$ M both in the middle of the range for sole effect on carbonic anhydrase as stated by Maren (1977). No effect of acetazolamide other than carbonic anhydrase inhibition is known for concentrations below 10^{-3} M (Maren 1977).

It was found that after systemically administered acetazolamide cornea guttatae did not retain the deswelling capacity as seen in the control group. Considering the fellow non-operated guttata eye a small swelling tendency was also noted. A tendency towards an IOP-drop however was seen in this case. Normal corneas were not affected by acetazolamide. The reason for this could be several. Differences in IOP may be ruled out (Fig. 2). Becker (1957) and Maren (1976) found only small changes in the aqueous plasma ratio (already nearly one) of pH , Cl^- , HCO_3^- and Na^+ after acetazolamide medication changes so small that they could not explain the loss of capacity for corneal deswelling (Hodson 1971, Dikstein & Maurice 1972).

Differences in aqueous concentration of acetazolamide between normal and guttata eyes cannot be completely ruled out. The relative permeability of the ciliary epithelium of cornea guttata eyes to acetazolamide is not known neither in the normal state nor when modified as after cataract surgery. Further in the actual region of drug-concentration (10^{-5} M) the physiological response exhibits a step course so that small changes in concentration would yield greater physiological response.

It seems tempting to speculate that corneae guttatae are in a state close to decompensation considering dehydration. The uncatalyzed hydration of CO_2 in aqueous is so fast ($t = 0.5$ sec Silverman 1973) that only when corneal dehydration is almost decompensated could an effect of carbonic anhydrase inhibition be expected. Fuchs endothelial dystrophy exhibiting corneal guttae is a condition with frank oedema. The carbonic anhydrase dependence of the pump was found to be 33–60% (Fischbarg & Lim 1974, Hodson & Miller 1976, Hull et al 1977) so partly inhibiting an active maybe nearly decompensated fluid pump may be the straw that broke the camel's back.

The post-operative CCT-course of the group guttata control is seen to follow the two normal groups for the first 3 days. Then a secondary rise in thickness is noted also found by Olsen (1980). After penetrating keratoplasty Branssen & Ehlers (1979) found that not until 3 days after operation was CCT at maximum value if corneal host disease as Fuchs endothelial dystrophy suggesting some aqueous factor to be involved in the regulation of CCT.

This study would seem to contradict the use of acetazolamide in the treatment of Fuchs endothelial dystrophy (Warring et al 1978).

So the results in this clinical study on the human cornea would be consistent with earlier studies on rabbit corneas in vitro that fluid transport across corneal endothelium at least partly is based on carbonic anhydrase supplying bicarbonate to a transport system.

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SEMIQUANTITATIVE CYTOLOGIC ANALYSIS OF FLUID WASHED THROUGH THE LACRIMAL PASSAGE

BY

M S NORN

A quantitative cytologic analysis of the sediment of 3 ml of saline (0.9%) washed through the lacrimal drainage system of 95 normal and 84 diseased eyes revealed that the cytological picture reflects the conditions in the lacrimal passages and not in the conjunctiva

Neutrophilia was seen to become increasingly frequent with slowing down of the flow through the lacrimal passage estimated on the basis of dye dilution in the lacrimal river and dye dilution in the fluid washed through the lacrimal drainage system and also by the spontaneous passing of the dye to the nasopharynx

Neutrophilia was present in no more than 8% of normal eyes as against 69% of eyes with epiphora 83% with infectious conjunctivitis 40% with conjunctivitis sicca and 50% with chronic simple conjunctivitis

Neither lymphocytosis nor eosinophilia was seen in any normal eyes

Erythrocytes were admixed artificially by washing through and probing

Granules were absent in normal eyes but present in 5% of the diseased eyes while hairs were found in 4 and 14% respectively and the fungus *Alternaria* in 4 and 1%

Key words cytology conjunctiva lacrimal drainage system - epiphora - vital staining rose bengal - neutrophilia

The cytology of the conjunctival fluid can be studied quantitatively e.g. by suction through a pipette laterally in the inferior fornix over an area of 3.14 mm² (Norn 1960). This can give us valuable clinical information. Thus over 100 neutrophilic granulocytes indicate presence of bacterial conjunctivitis over 50 columnar epithelial cells pathologically increased epithelial desquamation etc.

A similar cytologic analysis of the lacrimal sac and duct seems to be worth while. Therefore, after washing through of the lacrimal passage the wash fluid was examined for amounts of cells. The aim was 1) to examine a normal material, 2) to examine a clinical material (epiphora among others), 3) to ascertain whether the cell numbers corresponded to those of the conjunctival fluid, in other words, whether the cells had merely passed from the conjunctiva into the lacrimal passage or the cells had been formed locally, thus differing from those of the conjunctiva in type and number.

Method

Washing through of the lacrimal passage was preceded by the following clinical examinations: break up time (stability of the precorneal film estimated using a stop watch after vital staining by 0.125% fluorescein). Then a mixture of rose bengal and fluorescein (1% of each) was instilled. The dye dilution in the lacrimal river indicating the tear flow was estimated after exactly 5 min (lacrimal river dilution test). Rose bengal staining was scored on cornea and conjunctiva according to a score system marking the degree of possible drying up (up to 30 scores gave one point, less than 100 two points, less than 1000 three points, less than 10 000 four points and more than 10 000 five points in exposed temporal and nasal segments of the conjunctiva and on the cornea, i.e. maximally 15 points per eye). Rose bengal vital staining along the lid margin (Marx line) and its relation to the punctum lacrimale was noted down (ectropion, entropion, deficient functioning).

After 10 min. and again after another 10 min. spontaneous passing of dye to nose and pharynx was controlled by Wood's light built into a magnifying glass. In some cases following repeated intense blowing of the nose and examination of the handkerchief used.

Residual vital stain was removed from the conjunctival sac by washing with salt and an anaesthetic (0.4% oxibuprocaine) was instilled. Then the lacrimal p. was washed through with saline, if necessary after dilation of the punctum lac. by a conical probe.

The washing through was performed through a thin blunt angular mounted on a syringe containing 5 ml of 0.9% saline. The patient's head so far forward that the wash fluid could pass direct into a kidney shaped patient's mouth was kept closed. In cases with obstructed passage performed and thereafter washing.

The wash fluid was centrifuged (1000 rpm for 5 min). The sediment a drop of 10% formaldehyde dried on a slide and afterwards stained with fuchsin and eosin.

Regarding the individual methods including cell counting technique and mucus grading reference is made to Norm (1974-1977)

Student's *t* test was employed for statistical calculation of the *P* value

Material

A total of 109 lacrimal passages from 59 subjects were examined (30 females and 29 males ranging in age from 14 to 80 mean age 53.7)

The normal material comprised 25 lacrimal passages from 25 subjects 12 females and 10 males ranging in age from 20 to 80 mean age 49.3

The pathological material was composed as shown in Table II. The 22 persons (32 eyes) with epiphora were all without rhinitis. The epiphora was unilateral in 11 persons, one side predominated in 7 and the resting 4 cases were equally bilaterally.

Results

The cytologic sample of wash fluid from the lacrimal passage was of a reasonably good quality being suitable for estimation of cell types, cell numbers and contents of mucus.

Table I
Cell numbers in fluid washed through the lacrimal passage in normal eyes

	Number of cells				Number of lacrimal passages above upper limit (in %)
	Average	Range	10-90% percentile	normal upper limit	
Neutrophilic granulocyte	38	1-390	5-50	100	(8)
Lymphocytes	3	0-20	0-8	100	(0)
Nucleated squamous cells	13	0-93	0-28	50	(4)
Keratinised squamous cells	2	0-30	0-4	50	(0)
Cuboid epithelial cells	7	0-9	0-14	50	(4)
Columnar epithelial cells	15	0-8	0-	50	(0)

Table II

Cell numbers in fluid washed through the lacrimal passage ■ diseased eyes Average numbers of cells and in brackets percentage of lacrimal passages above upper normal limit (cf. Table I)

Diagnosis	Neutrophilic granulocytes	Lymphocytes	Nucleated squamous cells	Keratinised squamous cells	Cuboid epithelial cells	Columnar epithelial cells	Number of lacrimal passages
Dacryocystitis	125 393 (88)	11 393 (25)	6 (13)	0	8 (0)	8 (0)	8
Dacryocysto-rhinostomy	4 454 (100)	142 (40)	120 (50)	8 (0)	38 (0)	0	5
Ephiphora	12 641 (69)	108 (25)	476 (50)	529 (15)	238 (38)	122 (9)	32
Entropion	21 (0)	2 (0)	3 (0)	0	2 (0)	3 (0)	4
Entropion or lacking punctum lacrimale	20 580 (100)	2110 (67)	93 (33)	0	0	0	3
Conj. sicca	283 (40)	106 (30)	222 (50)	34 (10)	99 (40)	20 (10)	10
Conj. simplex	824 (50)	31 (13)	103 (50)	19 (13)	59 (25)	7 (0)	8
Conj. infect.	100 (83)	408 (67)	313 (67)	13 (17)	50 (37)	0	6
Others*	1741 (38)	12 (0)	164 (50)	53 (13)	11 (13)	1012 (38)	8

* Others comprise papillomatous conj (2) follicular conj (2) corneal transplants 30 (3) and facial nerve palsy (2)

Normal eyes

The findings regarding cells in normal eyes are shown in Table I. Generally only few cells were present but all the samples were found to contain some cells. The lowest total number was three.

Neutrophilic granulocytes were noticed in all cases on an average 38 cells. Only two samples had more than 100 cells (320 and 120 respectively).

Lymphocytes were rarer being present in 52% most often in fairly small numbers.

Epithelial cells were of the same types as in the conjunctiva: cuboid epithelial cells (in 44%), columnar (in 40%), nucleated squamous cells (in 68%) and keratinized nucleated squamous cells (in 24%). The various cell types were practically always present in numbers under 50. In a few cases the columnar cells were longer than they generally are in the conjunctiva.

Table I shows the upper cell number limits to be equal in quantitative pipette specimens from the conjunctiva and in the fluid washed through the lacrimal passage: namely 100 for neutrophilic leucocytes and lymphocytes and 50 for each of the epithelial cell types, while eosinophilic granulocytes were absent in normal eyes.

Flakes of epithelial cells with coherent cells occurred in 24%. Only one case (4%) had a total of over 50 cells in one sample (28 cuboid, 24 squamous and 20 columnar).

Erythrocytes were seen in 64%, less than 100 erythrocytes in 44% (innumerable (millions) in 8% of the 25 normal eyes). The washing may thus in some instances proceed without injuring blood vessels even when a conical probe is used while in others profuse haemorrhage occurs.

Table III

Neutrophilia in lacrimal passage in relation to tear flow test, 109 cases. The figures represent percentages of eyes with neutrophilia. Normal lacrimal river dilution test defined as dilution to at least a pale orange colour within 5 min. Fluid washed through the lacrimal passage is normally yellow while a more intense colour (orange-red, $N=12$) and no colour at all ($N=13$) indicate pathological conditions.

	Normal	Pathological	P
Lacrimal river dilution test	43	64	< 0.05
Spontaneous passing of dye to nasopharynx	41	64	< 0.05
Dye in wash fluid	40	62	< 0.05

No granules were found in the wash fluid. In one case (4%) coiled hairs were noticed (16 with roots presumably cilia) while fungi (seven *Alternaria* specimens without neutrophilia) occurred in one case only (4%).

Diseased eyes

Neutrophilia (more than 100 neutrophilic granulocytes predominating over lymphocytes) suggests bacterial infection of the conjunctiva (Norm 1974). Neutrophilia was very frequent in the fluid washed through the lacrimal passage (Table II) against in no more than 8% of the normal eyes (Table I).

As might be expected, neutrophilia in the lacrimal passage was frequent and very pronounced in cases of dacryocystitis. There were up to nearly one million cells a veritable lump of pus.

Neutrophilia was also seen after Toti's operation indicating persisting bacterial infection in the system or nose. Epiphora was associated with neutrophilia in 69% (cf. 8% of normal eyes, $P < 0.001$). There was pronounced neutrophilia in ectropion and failing punctum lacrimale function, whereas not so in entropion.

Table III illustrates the relation between occurrence of neutrophilia and results of lacrimal river dilution tests in the total material. Neutrophilia proved to be most frequent in relation to reduced tear flow, obstructed spontaneous passage of dye to nose and pharynx, and non-dilution of dye instilled into the conjunctiva and afterwards examined in the fluid washed through. The same tendency prevailed for the group of isolated epiphora. On the other hand, no correlation was noticed between occurrence of neutrophilia in fluid washed through the lacrimal drainage system and break up time or rose bengal vital staining of cornea and conjunctiva.

This goes to show that impeded passage through the lacrimal drainage system is attributable to neutrophilia in the region concerned.

In infectious bacterial conjunctivitis neutrophilia may be present not only in the conjunctiva but also in the lacrimal passage. The latter is not pronounced, however, nor is it always present.

Conversely, neutrophilia in the lacrimal passage was often seen in chronic simple conjunctivitis and keratoconjunctivitis sicca without conjunctival infection.

Eosinophilic granulocytes were not detected in the tear fluid.

Lymphocytes were frequently found in fairly large numbers, most often concurrently with neutrophilia. Proper lymphocytosis (more than 100 lymphocytes predominating over neutrophils) was demonstrated in the lacrimal passage in one case only (a case of keratoconjunctivitis sicca).

Epithelial cells in increased numbers were frequently observed in association with neutrophilia in the lacrimal passage. All types were surprisingly often present in pathological numbers within the epiphora group compared with proper dacryocystitis, where neutrophilic granulocytes predominated.

In no more than six cases were pathological numbers of epithelial cells noticed without co-existing neutrophilia. These were scattered over the various diagnostic groups (conjunctivitis sicca, papillomatous conjunctivitis, follicular conjunctivitis). Their occurrence seemed to be independent of probing difficulties.

Epithelial cell flakes and erythrocytes occurred in approximately the same numbers in the individual groups as in normal eyes.

Fungi In 5% bluish black coarse granules were seen together with neutrophilia and in one of these also *Alternaria* (fungus specimens). In one case (sicca) a lump was observed consisting of a thousand anuclear squamous cells.

Hairs were detected in the wash fluid in 14% distributed over all diagnostic groups, maximum 30 hairs in one sample. This finding does not differ significantly from that in the normal material. In the pathological material showing hairs the number of neutrophils was not higher than might be expected (67% with neutrophilia. Average number of neutrophils per sample 7.459 against total pathological material 13.967).

Relation to cytology of conjunctiva

The cytology of the conjunctival fluid of 80 eyes was analysed quantitatively before the lacrimal passage was washed through. Neutrophilia was found in the wash fluid in 45% with conjunctival neutrophilia and 49% without (Table IV).

Neither was any correlation ascertained between the cell contents in the conjunctival fluid and the lacrimal passage as regards eosinophilia (three had conjunctival eosinophilia while none had lacrimal passage eosinophilia), lymphocytosis (three had conjunctival lymphocytosis *alone* and one lacrimal passage lymphocytosis *alone*), increased number of epithelial cells or mucus.

Table IV

Relation between neutrophilia in conjunctiva and lacrimal drainage system estimated by quantitative cytologic pipette sample test from the conjunctiva (Norn 1974) and quantitative analysis of fluid washed through the lacrimal passage. A total of 80 eyes.

	Lacrimal passage	
	Normal	Neutrophilia
Conjunctiva normal	24	23
Conjunctiva neutrophilia	18	15

Colour and mucus content of fluid washed through lacrimal passage

The colour of the wash fluid was studied in the slit lamp in white light and in cobalt filtered light. In 49% of the total material of 109 eyes the colour was yellow and in 39% orange or red, indicating a slower passing during the experimental period. In the remaining 12% the fluid was colourless (failing transfer of rose bengal fluorescein vital stain from the conjunctiva to the lacrimal passage).

Neutrophilia in the lacrimal passage was seen to be increasingly frequent the more impeded the flow: 40, 60 and 69% in the stated order (cf. Table III).

The amount of mucus in the fluid washed through the lacrimal passage was likewise seen to increase the slower the flow. Thus grade 3 mucus was found in 11% with yellow wash fluid, 59% with fluid of a stronger colour and 54% with colourless fluid ($P < 0.01$).

The amount of mucus was increased in dacryocystitis (average grade 3.4 against the normal 1.8), epiphora (2.4), ectropion (3.3), dacryocystorhinostomy (2.8) but normal in simple conjunctivitis (1.8) and conjunctivitis sicca (1.4).

Flocculent particles, sediment or milky wash fluid was found in 32 of the 109 eyes. This indicates admixture of blood (more than 1 million erythrocytes in 34% against 0% in clear wash fluid, $P < 0.001$) or neutrophilia (in 72% against 49% in clear wash fluid, $P < 0.02$). The amount of mucus in the lacrimal passage seemed to be increased (mean grade 2.64 against 2.17 in the total material).

Discussion

The cells in the fluid washed through the lacrimal passage might be concerned simply to reflect the cytologic conditions in the conjunctival fluid. Cells from the conjunctiva are to pass through the lacrimal passage just as instilled vital stain for instance.

The present investigation was the first to demonstrate by a quantitative cytologic technique that the cytologic conditions in the two regions are in the main independent of each other. An analysis of the cytology of the lacrimal passage can therefore give information of clinical value. The normal figures for the wash fluid happened to correspond to the figures arrived at in the conjunctivo-cytologic analysis, though the areas differed (the hollow space of the lacrimal passage and part of the nose versus 3 mm² conjunctival area).

A reduced flow will raise the cell number in the lacrimal passage despite an unchanging cell production, the cells being removed at a slower rate. However this cannot alone explain the great variations noticed in the present study, because some cases were seen with very few cells despite arrested flow, and others with many cells

despite unimpeded flow and passage. The percentage distribution of cells also varied appreciably.

The most important cytologic finding is that of neutrophilia. The clinical material showed the degree of neutrophilia to rise with decreasing tear flow. Neutrophilia indicates bacterial infection. One gets the impression that repeated infections in the lacrimal passage cause neutrophilia which will impede the flow. This gives rise to bacterial re-infection with neutrophilia resulting in further slowing down of the flow. Moderate epiphora will thus proceed to becoming pronounced epiphora which perhaps gradually will develop into proper dacryocystitis.

In assessing the wash fluid sample cytologically we must therefore attach importance to the finding of neutrophilia which indicates bacterial infection of the lacrimal drainage system. Antibiotic treatment by washing (or conjunctival instillation) is recommended.

Finding of fairly large granule formations suggest presence of tear stones (fungi). Large amounts of epithelial cells may presumably obstruct the passage through the system. We can hardly attach any importance to observation of hairs, the fungus *Alternaria* or erythrocytes (cf. findings in normal eyes: mistaken hairs from the nose and regarding erythrocytes: mechanical damage by washing).

Examination of the wash fluid may be of value even without cytologic analysis provided it has been preceded by rose bengal fluorescein vital staining of the conjunctiva.

Dilution of the wash fluid to yellow colour indicated good function, to orange or red colour impeded flow, and no colour occluded lacrimal drainage system. Flocculent particles suggest neutrophilia or admixture of blood.

Cytologic analysis gives further details. Larger clinical materials can clarify the significance of lymphocytosis (cf. Coster et al. 1979) and eosinophilia in the lacrimal drainage system.

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HLA ANTIGENS IN CASES OF GIANT CELL ARTERITIS

BY

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HLA tissue type antigen determination for A, B and C antigens in 88 patients suffering from giant cell arteritis of the temporal artery showed no significant deviations as compared to a control material of 3164 blood donors. A weak indication of association with antigen HLA B8 appeared to be of interest due to a corresponding indication in a previous investigation. The patients were a mixed hospital material consisting of cases of clinical temporal arteritis and patients with polymyalgia rheumatica. There was an overrepresentation of women (77%). Familial occurrence was demonstrated sporadically (3 pairs of siblings).

Key words: giant cell arteritis - temporal arteritis - polymyalgia rheumatica - HLA antigens - familial occurrence.

The etiology of giant cell arteritis is unknown. An immune reaction is a possibility (for a survey see Hunder & Allen 1978). The HLA antigenic determinants in the cell membranes are considered as being related to cell recognition and immunological response (Bodmer 1972). In addition it is considered that the genes of

chromosome No. 6 are closely linked to immune response genes. Population studies regarding association between HLA antigens and immune related diseases are therefore of interest. Terasaki et al (1976) and Hunder et al (1977) were unable to demonstrate association in 9 and 43 patients with giant cell arteritis respectively. Seignalet et al (1977) found an increased occurrence of HLA B14 in 61 patients ($P = 0.002$) while Hazleman et al (1977) found that HLA B8 occurred more frequently in 30 patients suffering from giant cell arteritis ($P = 0.02$) as well as in 27 patients with polymyalgia rheumatica ($P = 0.002$) where the artery biopsy was negative.

Material

The material comprises 88 unrelated patients (66 women ages 58-100 average 75 years and 22 men ages 57-87 average 75 years). These patients had been admitted to a Danish hospital either the medical or ophthalmological departments during the period 1974-1979. Histological examination of the temporal artery or one of its branches revealed arteritis compatible with the diagnosis giant cell arteritis (histopathological criteria as described by Hunder et al (1975)).

Nineteen patients had the clinical diagnosis polymyalgia rheumatica where a biopsy had demonstrated giant cell arteritis of the temporal artery. Sixty nine patients had clinical symptoms of temporal arteritis. Unilateral severe reduction in vision was a symptom in 18 patients while this was present in both eyes in a further four. In 59 patients a diagnosis of giant cell arteritis was made during admission to a medical department and in 29 patients in the ophthalmological department.

Method

Blood samples were withdrawn during admission to hospital or in the outpatient clinic. In a number of cases the patients were requested to attend the clinic for blood sampling and in seven other cases these were withdrawn at home. The results of the HLA typing were compared to those of a control material consisting of 3164 blood donors (1016 donors for C antigens). HLA type determination was carried out according to the method of Kussmeyer, Nielsen & Kjerbye (1967). Two \times two tables for the individual antigens were evaluated by means of Fisher's test (one sided).

Table I

Frequency of phenotypes in patients and the control population Giant cell arteritis (N=9)
Control (N=3164) *P* (Fisher)

HLA antigen	Number	%	Number	%	
A1	27	30.7	993	31.4	0.496
A2	49	55.7	1690	53.4	0.378
A3	27	30.7	870	27.5	0.91
A9	10	11.4	554	17.5	0.089
A10	11	12.5	286	9.0	0.179
A11	3	3.5	331	10.5	0.015
A28	6	6.8	278	8.8	0.340
Aw19	25	28.4	595	18.8	0.000
B7	12	13.6	349	10.8	0.945
B7	21	23.9	911	28.8	0.188
B8	28	31.8	731	23.1	0.041
B12	14	15.9	794	25.1	0.079
B13	5	5.7	133	4.2	0.317
B14	2	2.3	123	3.9	0.334
B18	6	6.8	234	7.4	0.593
B27	13	14.8	266	8.4	0.035
Bw15	13	14.8	566	17.9	0.976
Bw16	5	5.7	165	5.2	0.491
Bw17	2	2.3	259	8.2	0.968
Bw21	1	1.1	104	3.3	0.915
Bw22	2	2.3	111	3.5	0.403
Bw35	9	10.2	414	13.1	0.974
Bw40	24	27.3	579	18.3	0.097
(N=1016)					
Cw1	3	3.5	49	4.8	0.399
Cw2	15	17.0	96	9.4	0.094
Cw3	33	37.5	346	34.1	0.993
Cw4	8	9.1	139	13.7	0.145

Results

Table I shows the results. Five antigens (HLA Aw19 -B8 -B27 -Bw40 and -Cw2) showed increased values in the present patients ($0.05 > P > 0.01$) while two antigens showed decreased values (HLA A11 and -B12). If the *P* values are corrected by multiplication by the number of studied properties (27) then the differences are not significant.

The first patient to be studied was one of a monozygotic pair of twins both of whom suffered from giant cell arteritis (Kemp 1977 the twins are only counted as one patient in the present material). Further two cases of familial occurrence of the disease complex were observed. A 77-year-old woman included in the present material had a sister (deceased) who had developed severe temporal arteritis with bilateral loss of vision some seven years earlier. One 70-year old woman also included in the material had an older sister with polymyalgia rheumatica but no histologically verified arteritis. Information was obtained regarding the above relatives from the hospital to which they had been admitted.

Discussion

HLA B8 has previously been demonstrated as occurring more frequently in patients with temporal arteritis and polymyalgia rheumatica. The International HLA and Disease Registry in Copenhagen has incorporated the present data in a new combined analysis (1979). It was found that for HLA B8 in 222 patients with giant cell arteritis (the 9 patients of Terasaki et al. are not included) there was a relative risk of the disease in the antigen bearers equalling 1.56 ($P = 0.003$). Svegaard & Ryder (1977) state however that the significance limit in connection with combined analyses should be as low as 0.0004 in order to avoid false associations. Thus it is only an indication.

The present material includes 19 patients with the clinical diagnosis of polymyalgia rheumatica. It was impossible to determine retrospectively how many of the patients with clinical temporal arteritis suffered initially from symptoms of polymyalgia. Both of the diseases are in all probability cardinal manifestations of a generalized giant cell arteritis (Sørensen & Lorenzen 1977).

The frequency of giant cell arteritis in the Danish population is increasing (Kemp 1977) but awareness of the disease has also increased since 1934 (Horton et al. 1934). Classical hereditary studies are not possible as the disease has only been registered for the last 40 years. Further very few suffer from the disease before the age of 50 years. Liang et al. (1974) have in connection with their report of four pairs of closely related patients suggested that there are unknown genetic factors which predispose elderly individuals to polymyalgia rheumatica and temporal arteritis. Up to the present it has been impossible to demonstrate any definite connection between such factors and the HLA system.

Acknowledgment

The authors wish to thank the medical and ophthalmological departments in Esbjerg and Høding as well as the medical departments of the hospitals in Faborg, Odense and Ribe all of which have kindly put patients at the disposition of the authors for study.

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THE COLOUR OF THE OPTIC DISC VARIATION WITH LOCATION OF ILLUMINATION

BY

PER NELLEMANN SØRENSEN

The variation of the colour of the optic disc was examined with a slit lamp and contact lens in 200 normal subjects. By moving the image of the slit across the optic disc this event was photographed in 15 subjects and colour densitometry was performed. The temporal part of the pale central excavation changes colour to red when illuminated nasally and vice versa.

The colour change could not be provoked in subjects with normal discs with flattening of the temporal disc sector. The whole neuroform reddens the whole way round when the slit hits the optic disc border and dependent on fundus pigmentation slight redness was produced even when the slit was placed outside the disc in the neighbourhood of the border.

Key words: central excavation - colour change - microdensitometry - optic disc colour

The colour of the optic disc is still an important clinical landmark in ophthalmology since it is explicitly correlated to the visual function (Cogan 1966, Walsh & Hoyt 1969). It is well known that the colour of the optic disc changes with the colour temperature of the light source (Miller & George 1978) and the age of the lens (Said & Weale 1959, Snyder 1964). It is also known that colour changes with the incident angle from the light source. Since the importance of this fact is only briefly mentioned in the literature (Kronfeldt 1967, Schwartz 1973) we have studied this phenomenon with the aid of a slit lamp and a contact lens simply by moving the image of the slit across the optic disc. In 15 cases photographs of this procedure were analyzed in a densitometer.

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Materials and Methods

200 normal subjects (age 6–87 mean 42 refractive error less than ± 3 d opt) had both of their optic discs examined in the Haag Streib or Rodenstock slit lamp employing a Goldmann fundus contact lens. When the image of the slit was moved horizontally or vertically across the disc the colour (redness) of the illuminated area and of the rest of the disc was noted as well as the point at which the disc first began to redden when the slit was moved towards and across the border.

Fifteen cases with cup disc ratio from 0.2 to 0.5 (estimated by colour contrast) were photographed (Zeiss photostill lamp magnification $\times 2$ Flash setting 1/300 sec slit 0.5 mm aperture 32 slit 3 Kodachrome 64). The transmission density of the diapositive was measured on the temporal slope of the optic disc exactly corresponding to an area with diameter 100 μ . The density was read on a Reichert microdensitometer in the green (540 nm) and red (640 nm) locus where the luminosity is highest and where the unwanted absorption from overlapping between the colour forming pigments is lowest. The density in the blue was not measured since it could be demonstrated in white areas only. The differences between the densities at 640 nm and 540 nm is taken as an arbitrary index of the red colour. The density at 640 nm denotes the colour hue and the density at 540 nm denotes the correction factor for variation in exposure.

Thus linearity exists between density and the logarithm to the exposure in the proper exposure range of the film for both green and red and furthermore the characteristic curves for green and red have practically identical slopes. Variation in exposure will therefore affect the densities in the same direction. The linearity requirement is not totally fulfilled in the bright parts of the film a fact that is considered of minor importance.

Results

Slit illumination of the optic disc results in a ring of hyperaemia in the peripheral part of the non excavated area. This happens as soon as the slit image hits the optic disc.

In most subjects (92%) also the central physiological cup is illuminated on the contralateral side of the optic cup. Thus illumination is temporally and vice versa. Most effective was due to the larger area of the optic cup hyperaemia on the nasal side.

In 6% of the subjects the excavation only was illuminated temporally. These subjects had temporal nasal displacement of the vessels.

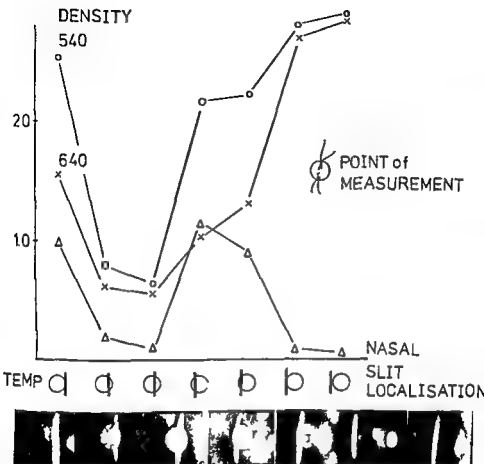


Fig 1

The redness of the central excavation as an illuminated slit moves stepwise across the left optic disc. Black and white prints of the colour diapositives in which density measurements was made is seen below. The red \times — \times (640 nm) and green \circ — \circ (540 nm) density is given (arbitrary units). The difference Δ — Δ is an index of the redness.

In all subjects the red glowing could also be provoked by moving the slit in the vertical direction.

No difference between left and right eye could be demonstrated in any subject.

In 35% of the subjects the optic disc begins to redden before the slit image reaches the border. These subjects were predominantly blonds with sparse fundus pigmentation. Thus 41% of the optic discs in blonds reacted in this way compare

Materials and Methods

200 normal subjects (age 6–87 mean 42 refractive error less than ± 3 diopters) had both of their optic discs examined in the Haag Streit or Rodenstock slit lamp employing a Goldmann fundus contact lens. When the image of the slit was moved horizontally or vertically across the disc the colour (redness) of the illuminated area and of the rest of the disc was noted as well as the point at which the disc first begins to redden when the slit was moved towards and across the border.

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Results

Slit illumination of the optic disc results in a light spreading effect of the whole neurorim i.e. the peripheral part of the non-excavated part of the optic disc. This happens as soon as the slit image hits the optic disc border.

In most subjects (92%) also the central physiological pallor turns red on the contralateral side of the optic cup. Thus illumination nasally causes reddening temporally and vice versa. Most effective was disc illumination from the nasal side due to the larger area of the optic cup and perhaps due to a more bank like neurorim on the nasal side.

In 6% of the subjects the excavation only glows when illuminated nasally but not temporally. These subjects had temporal flat bright but normal optic discs with nasal displacement of the vessels.

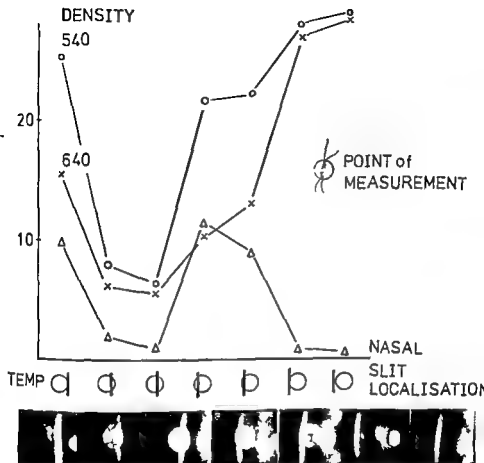


Fig 1

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In all subjects the red glowing could also be provoked by moving the slit in the vertical direction.

No difference between left and right eye could be demonstrated in any subject.

In 30% of the subjects the optic disc begins to redden before the slit image reaches the border. These subjects were predominantly blonds with sparse fundus pigmentation. Thus 41% of the optic discs in blonds reacted in this way compared



Fig 2

Slit lamp photo of the optic disc. The central excavation is white at the temporal side but red at the nasal side



Fig 3

Slit lamp photo of the same optic disc. The central excavation is now red temporally. No light spreading outside the optic disc is seen

to 19% of the optic discs in dark haired. However the reddening was always most pronounced when the slit image reached the border of the neurorim.

Fig 1 demonstrates the colour change of the temporal part of the optic cup when the slit image is moved across the optic disc in a typical record of a subject with well developed optic cup. The mean redness of the temporal excavation when the slit image is placed on the nasal neurorim is 0.96 (range 0.71-1.10 $n = 15$). The redness is given by the numerical difference between the densities of the film at 640 nm and 540 nm (arbitrary units).

From Fig 1 it is seen that the optic cup reddens by slit illumination both on the temporal border and also by nasal illumination.

Fig 2 and 3 are colour prints of diapositives of the colour change of the central excavation when the slit image is placed temporally (Fig 2) and nasally (Fig 3). Light spreading in the retina is minimal compared to that of the optic disc.

Discussion

This investigation has shown that the optic disc colour depends on the location of the incident light on the disc. The demonstrated colour change of the physiological excavation of the disc from pale to red when the slit image is moved across the disc is puzzling. Quigley & Anderson (1977) in a histopathological study on monkey eyes with descending optic atrophy considered the optic nerve fiber bundles and their adjacent glial columns as optic pathways. According to them light is transferred to the optic disc longitudinal to the nervous fibres and adjacent glial cells and later diffused among the capillaries from which the reflected light takes its pink colour. The contralateral reddening of the central excavation however is difficult to explain in this way. On the other hand Gloster (1972-1973) in his study of optic discs onto which a grid shadow was projected through a fundus camera explains the light transfer of the optic disc tissue as an effect of a diffusing of light projected onto it.

Still the nature of the colour change from pale to red of the contralateral part of the central excavation is obscure. This phenomenon might be either a guidance or a reflection of red light originating from the vascular structures of the disc tissue. Another possibility could be that the colour change is due to an unveiling of underlying vascularity hitherto hidden by veiling reflections from tissue in front of it, just as the vascularity of the central excavation and apparently pale temporal parts of the optic disc is disclosed by fluorescein angiography (Hayreh 1969-1972).

When light reaches a borderline surface two possibilities exist dependent on the angle of incidence. Light can either be reflected or refracted. Reflection can be

specular or scattered according to the properties of the surface. In transparent media refractive index differences determine the degree of refraction. Thus the redness of the optic disc must depend on whether light is scattered or reflected from the surface or penetrates the tissue until it is absorbed or finally reflected from the lamina cribrosa.

The optical properties of the optic disc are not known in detail and need further investigations.

The described dependence of optic disc colour on different location of the illumination of the disc has great clinical importance. Schwartz (1973) advocates examination of the optic disc to be done with the aid of a contact lens and the slit lamp. For example with the direct ophthalmoscope the optic cup is estimated larger.

Better understanding of the colour of the optic disc colour is achieved when considering the light reflected from the optic disc tissue as an information on what transformation the light undergoes when it strikes the optic disc texture.

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PULSED DOPPLER ULTRASOUND FOR MEASURING BLOOD FLOW VELOCITY IN THE HUMAN OPHTHALMIC CIRCULATION

BY

SIGMUND KVERNES SIGMUND BLIKA JAN GILTVEDT KNUT MATRE
KJELL KRISTOFFERSEN ARNE GRIP and ALF O. BRUBAKK

This study was performed in order to develop a method for studying blood flow in the ophthalmic circulation. Using a pulsed doppler system utilizing an ultrasonic frequency of 10 MHz, blood flow velocities have been measured in the ophthalmic artery and in the arteries behind the eyeball (lateral posterior ciliary arteries) in 40 normal subjects. The mean of the peak systolic velocities were 34 ± 6 cm/s in the ophthalmic artery and 14 ± 3 cm/s in the lateral posterior ciliary arteries. We conclude that blood flow velocities can be measured in defined vessel areas in the orbit.

Key words: ophthalmic artery — posterior ciliary arteries — blood flow velocities — pulsed doppler ultrasound

Using fluorescein photographic and photometric techniques, blood flow in retinal arteries can be studied (Hickam & Frayser 1966; Niesel & Gassmann 1972).

Relative changes in blood flow to the eye in cases of carotid stenosis have been studied using ocular pulse amplitude (Høver et al. 1971). Only indirect measurement of blood flow can be performed in this way as changes in ocular volume and compliance will influence the result.

Methods based on continuous wave ultrasound and the doppler principle have been proposed for studying ocular blood flow (Tokoro 1972; Yamamoto 1975).

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Methods based on continuous wave ultrasound and the doppler principle have been proposed for studying ocular blood flow (Tokoro 1972; Yamamoto 1975).

These methods do not permit range resolution and therefore can not give a defined sample volume with respect to depth.

The method presented in this paper has been based on pulsed doppler ultrasound which permits range resolution and hence measurement of blood flow velocities of selected depths. Furthermore, the size of the sample volume has been experimentally determined. By this method it is possible to measure blood flow velocities more accurately in the ophthalmic and the posterior ciliary arteries.

In this study the feasibility of the method has been demonstrated and the reproducibility of the measurements as well as the range of normal velocities have been documented.

Methods

When ultrasound is transmitted into the body, the soundwaves are scattered from inhomogenities in the tissue and in the blood. The frequency shift (doppler shift f_d) in reflected waves is proportional to the velocity (v) of the scatterers according to the formula

$$f_d = 2 f_0 \frac{v}{c} \cos \Theta$$

where f_0 is the frequency of the transmitted soundwaves, c is the sound velocity in the tissue and Θ is the angle between the soundbeam and the path of the scatterers.

By pulsing the transmitted waves and detecting the backscattered soundwaves at a given time interval after transmission, depth resolution is achieved (Baker 1970).

The ultrasonic doppler velocity meter used in this study has been described in detail (Angelsen & Brubakk 1976) and only its main features will be given here.

The ultrasonic frequency is 10 MHz. The repetition frequency was 19.5 kHz and a pulse length of 3 microseconds gives a sample length of 4.6 mm. The ultrasonic transducer acts both as a transmitter and as a receiver. The backscattered ultrasound is split into two channels by a quadrature demodulator in order to separate forward from backward flow.

In order to remove signals from slowly moving tissues, a high pass filter with a cut off frequency of 290 Hz was used. The low pass filter has a cut off frequency of 9.3 kHz to remove high frequency noise.

Using analogue estimators described earlier (Angelsen & Brubakk 1976, Brubakk et al. 1977) the mean and maximum velocity in the sample volume can be displayed. However, due to the poor signal/noise ratio when measuring from the posterior ciliary arteries, analogue estimation is difficult and the received signals were analyzed using a frequency analyzer. This instrument is based on a z-chirp algorithm implemented by analogue shift registers (Reticon Corp., Sunnyvale, USA).

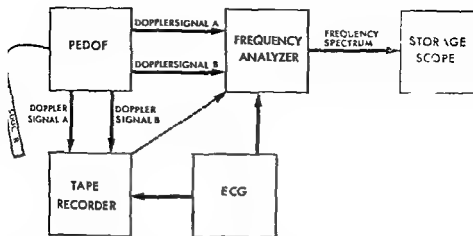


Fig 1

A schematic view of the signal processing equipment. PEDOF Pulsed Echo-Doppler Flow Meter is developed at the Division of Engineering Cybernetics University of Trondheim

In order to improve the time resolution of the received signals curves from about ten cardiac cycles were superimposed on an oscilloscope screen using the subject's electrocardiogram as a trigger. The maximum velocity has been given by the envelope around the frequency spectrum.

A schematic representation of the equipment used is shown in Fig 1.

Initially we tested a transducer fastened inside a gonioscopic lens hoping that this would facilitate aiming of the ultrasonic beam by simultaneous observation of the retina through a slit lamp. However this was not the case firstly because of an unavoidable pressure on the cornea secondly because of reduced mobility of the transducer. All measurements in this paper have therefore been done with a rod-shaped transducer made of lead zirconate-titanate (Trade Mark PZT 5-A Brush Clevite). The transducer is quadratic (3 mm) and is backed by araldite. The transducer has an acoustic output power of 28 mW/cm², an efficiency of 12%, a phase distortion of 2% and an impedance of 17Ω.

The output power of the transducer was measured by transmitting the ultrasonic beam into an absorber and detecting the ultrasonic energy by a sensitive balance weight (Mettler H. L. 52). The principle of this method had been described elsewhere (Kosoff 1965; Matre 1979).

The acoustic field from the transducer was measured in water (Engan 1978). The field was detected by another 10 MHz transducer having a small aperture (0.5 mm) in front of it. The rod transducer was scanned in the x-direction and in the

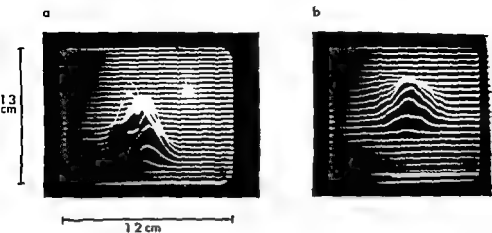


Fig 2

A three dimensional representation of the ultrasonic field of the rodshaped transducer in water. The field from the 10 MHz transducer is detected by scanning a 10 MHz rod transducer of same type with a 0.5 mm aperture in front in a plane 90° to the beam. a) 1 cm from the crystal b) 3 cm from the crystal

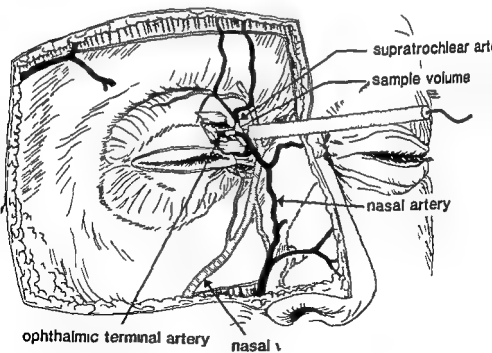


Fig 3

End branches of the ophthalmic artery. The or the sample volume is shown about 8 mm below

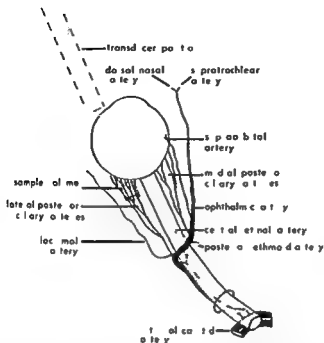


Fig 4

The most common pattern of some of the branches of the ophthalmic artery. The sample volume is located lateral to the optic nerve 24 to 28 mm behind limbus cornea corresponding to the lateral main bundle of the posterior ciliary arteries. The size of the volume is estimated to be 4.6 mm long and 4 mm in diameter. The direction of the transducer is indicated parallel to the optic nerve. Redrawn after Hayreh S S (1976)

y-direction simultaneously. Fig 2 shows a three-dimensional representation of the sound field in a plane 90° to the ultrasonic beam in water at two different depths.

Blood flow velocity was measured in 40 subjects with no history of cardiovascular or eye diseases: 16 women and 24 men (mean age 36 years, range 15–70 years).

Nobody was using any drug at the time of the investigation. The procedure was carefully explained to every subject and consent for doing the study was obtained. All measurements were performed with the subjects in a supine position from the following sites: Left and right ophthalmic artery and left and right arteries behind the eyeball (later called lateral posterior ciliary arteries).

In order to measure flow velocities in the ophthalmic artery, the transducer was positioned on the nasal part of the closed upper eyelid. Using methylcellulose as a coupling agent it was possible to measure without exerting any pressure on the tissue. The transducer was moved until maximum velocity was obtained.

The strongest signals were found at approximately 8 mm depth in all subjects. Probably this will occur just before the division of the ophthalmic artery into the supratrochlear and the dorsal nasal artery (Hayreh *et al.* 1962). The approximate location of the sample volume in relation to the vessels can be seen from Fig. 3.

In order to measure the velocities in the lateral posterior ciliary arteries eye movements must be eliminated; therefore the subjects were asked to focus a black spot in the ceiling. The cornea was anesthetized with oxybuprocaine chloride 0.4% eye drops. To avoid sound absorption in the cornea and in the lens the transducer was placed at the limbus cornea in a direction approximately parallel to the optic nerve axis (Fig. 4) and the position was changed until the strongest signals were detected, usually from a depth of 26 mm behind the corneal limbus.

Adequate signals, however, could be detected at depths from 24 to 28 mm. Using methylcellulose no pressure was needed to obtain acoustic contact with the eyeball.

In order to demonstrate changes in flow following alteration of the intraocular pressure, measurements were performed using different weights on the cornea (35–120 g).

Results

Typical velocity spectra from the common carotid artery, the ophthalmic artery and the lateral posterior ciliary arteries are shown in Fig. 5. Each spectrum is a superimposition of about 10 cardiac cycles from a normal subject, 30 years of age.

The peak systolic velocities found in the ophthalmic artery and the lateral posterior ciliary arteries in 40 normal subjects have been shown (Table 1).

The mean of the peak systolic velocities have been grouped according to age. No significant difference in maximum systolic velocities were found with increasing age. The form of the maximum velocity curve, however, changed with increasing age (Fig. 6).

In Fig. 7 histograms of the peak systolic velocities have been shown. The velocities ranged from 21 to 30 cm/s in the ophthalmic arteries and from 10 to 25 cm/s for the lateral posterior ciliary arteries.

In Table II the mean of the peak systolic velocities from each of 3 normal subjects measured 10 times, weeks apart, is presented.

Fig. 8 shows the effect on blood flow velocities in the lateral posterior ciliary arteries when intraocular pressure was changed. The velocity spectrum in Fig. 8a was recorded at normal intraocular pressure.

In Fig. 8b and 8c the intraocular pressure was increased for 30 seconds by placing an external weight, 35 g and 60 g, respectively, on the cornea. With increasing intraocular pressure the maximum flow velocities were reduced, especially its diastolic part. After release of the pressure (Fig. 8d) the velocity spectrum was

slightly increased as compared to Fig. 8a where normal flow conditions were to be expected. Weights of 90 g and 120 g were also tried. However, no flow could be registered in the posterior ciliary arteries.

In the ophthalmic artery the change of intraocular pressure led to no measurable velocity changes.

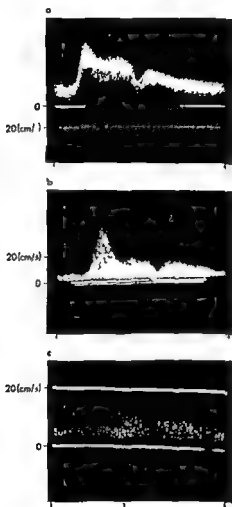


Fig. 5

Velocity spectrum averaged over about 10 cardiac cycles for a) the common carotid artery 13 mm below the skin surface b) the ophthalmic artery 8 mm below the skin surface c) lateral posterior ciliary arteries 96 mm behind limbus corneae

Table 1

Mean SD and variation coefficient of the peak systolic velocities in the ophthalmic arteries 8 mm below skin surface and in the arteries 26 mm behind the corneal limbus (lateral posterior ciliary arteries) from 40 normal subjects grouped according to age

No	Age range	Mean SD and variation coefficient of the peak systolic velocities (cm/s)			
		Ophthalmic artery		Lateral posterior ciliary arteries	
		Right side	Left side	Right side	Left side
9	(15-25)	36 ± 8	33 ± 8	16 ± 2	15 ± 3
8	(26-30)	34 ± 7	34 ± 5	13 ± 1	13 ± 1
8	(31-35)	32 ± 4	33 ± 4	14 ± 2	14 ± 1
7	(36-45)	37 ± 7	36 ± 6	14 ± 3	16 ± 5
8	(above 46)	35 ± 8	34 ± 8	14 ± 2	16 ± 4
40	(15-70)	34 ± 6	34 ± 6	14 ± 3	15 ± 3
		18%	18%	21%	20%
		22%	24%	12%	20%
		20%	15%	8%	8%
		13%	12%	14%	7%
		19%	17%	20%	31%
		22%	23%	14%	25%

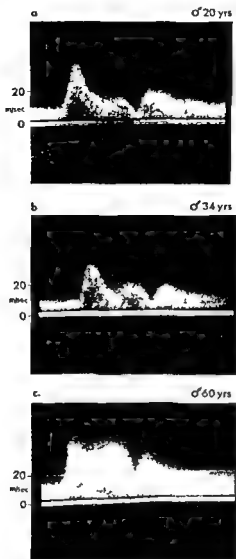


Fig 6

Velocity spectrum from the ophthalmic artery of normal subjects of different age a) male 20 years b) male 34 years c) male 60 years

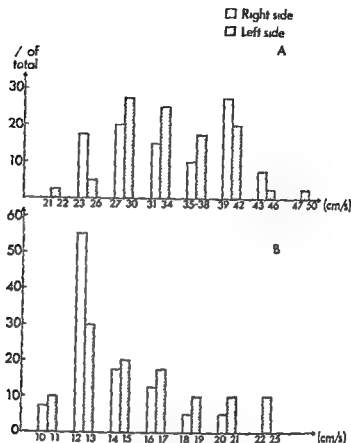


Fig 7

Distribution of the peak systolic velocities in A) ophthalmic artery B) lateral posterior ciliary arteries

Discussion

ing continuous wave ultrasound for measuring blood flow (Tokoro 1972 ...mamoto 1975) velocities in all vessels within the sound beam will be measured simultaneously. Our measurements were performed with pulsed ultrasound giving range resolution and thus the possibility of interference from undesirable vessels will be minimal.

In this study we have demonstrated that it is possible to measure blood flow velocities in defined parts of the ophthalmic arteries and in the posterior ciliary arteries reproducibly in normal persons.

Table II

Mean SD and variation coefficient of ten measurements from the ophthalmic artery 8 mm below skin surface and from the lateral posterior ciliary arteries for three individuals

Subject (Age)	Mean SD and variation coefficient of the peak systolic velocity (cm/s)				
	Ophthalmic artery		Lateral posterior ciliary arteries		
	Right side	Left side	Right side	Left side	
J C (28)	57 ± 4 13%	53 ± 3 9	128 ± 0.9 4%	131 ± 1.3 10%	
S B (38)	40 ± 2 5%	40 ± 1 3%	131 ± 0.9 7	116 ± 9.2 15%	
S L (30)	51 ± 3 10%	53 ± 4 10%	129 ± 1.0 8	129 ± 1.4 11%	

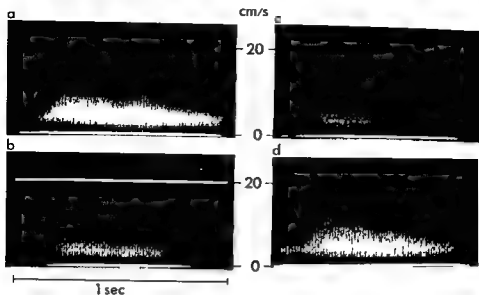


Fig 8

Velocity changes in the lateral posterior ciliary arteries following raised intraocular pressure achieved by placing different weights on the corneal surface for 30 seconds (Superimposition of about 10 cycles) a) Normal velocity spectrum b) 30 g c) 60 g d) after removing weights

The variation coefficients are rather great (20%) (Table I) and of the same order for the ophthalmic arteries as for the posterior ciliary arteries

Repeated measurements on the same person showed a variation coefficient of about 10% (Table II) The difference between the variation coefficients of these two groups is probably explained by anatomical variations

The angle between the blood stream and the position of the sample volume can not be known for certainty The posterior ciliary arteries consist normally of two main bundles one on the temporal and one on the nasal side of the optic nerve (Hayreh & Dass 1962 Hayreh 1962) With our procedure the angle between the sound beam and the blood stream will be small (Fig 4) and thus the influence on the measured velocities minimal An angle of $\pm 15^\circ$ for example would reduce the measured velocity only in the order of 3%

The course of the ophthalmic arteries shows greater variation Signal/noise ratio however is good and finding maximum signals corresponding to a minimum angle is easy

The sample area is given by the intensity of the sound which is proportional the field in the second power

The diameter of the effective sample area in our case will be less than 4 mm (F 2)

When measuring behind the eyeball with a cylindric sample volume with a length of 4.6 mm and a diameter of less than 4 mm the frequency spectrum of the backscattered sound will be a superimposition from several vessels. We have assumed that the main contribution will be from the lateral posterior ciliary arteries. Interference from other branches of the ophthalmic artery is possible. As the blood flow in the central retinal artery is very small compared to that in the ciliary arteries (Alm & Bill 1973) this contribution is probably negligible. Other branches of the ophthalmic artery will according to their anatomical course usually not interfere but may in case of vascular anomalies play a role.

We have measured the flow velocities in the terminal trunk of the ophthalmic artery and in the lateral posterior ciliary arteries at different intraocular pressure. This increase in pressure had no effect on the flow velocities in the ophthalmic artery. Changes in the velocities in the lateral posterior ciliary arteries however were considerable (Fig. 8). In particular the diastolic part of the cycle disappeared with only 30 g weight on the cornea.

The form of the maximum velocity curve from the small arteries like the ophthalmic artery and even from the lateral posterior ciliary arteries has been well preserved compared to the common carotid artery (Fig. 5).

The shape of the maximum velocity curve changed with increasing age showing a significant increase in the second systolic velocity peak in the older persons (Fig. 6). This increase is probably due to structural changes in the vessel wall.

With our instruments it is possible to measure velocities above 2.3 cm/s corresponding to the cut off frequency of the high pass filter which is necessary to remove noise from slowly moving targets.

When using ultrasound doppler to measure blood flow velocities uninvassively one problem is to reproduce the position of the sample volume from one measurement to the other. With knowledge about the topographical anatomy and an exactly known size of the sample volume as is the fact using pulsed ultrasound adequate reproducibility seems possible.

When using ultrasound for measuring in the orbit the possibility of damaging effects in the eye must be considered. So far no definite safety limits of ultrasound in ophthalmology have been determined. Experiments with pulsed ultrasound in rabbits (Barnett & Kosoff 1977) using a 7.5 MHz focused transducer with a mean power output of 160 mW/cm² on retina and 20 mW/cm² on the cornea for 30 min showed no morphological changes.

In this study the mean power into the eye was 28 mW/cm². Exposure time was kept as short as possible and did not exceed 15 min in any of the subjects. No adverse effects were observed.

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Table I

Frequency of phenotypes in patients and the control population Giant cell arteritis (N=88)
Control (N=3164) P (Fisher)

HLA antigen	Number	%	Number	%	
A1	27	30.7	993	31.4	0.496
A2	49	55.7	1690	53.4	0.378
A3	27	30.7	870	27.5	0.091
A9	10	11.4	554	17.5	0.089
A10	11	12.5	285	9.0	0.179
A11	3	3.5	331	10.5	0.015
A28	6	6.8	278	8.8	0.340
Aw19	25	28.4	595	18.8	0.070
B5	12	13.6	342	10.8	0.045
B7	21	23.9	911	28.8	0.188
B8	28	31.8	731	23.1	0.041
B12	14	15.9	794	25.1	0.009
B13	5	5.7	133	4.2	0.317
B14	2	2.3	123	3.9	0.334
B18	6	6.8	234	7.4	0.593
B27	13	14.8	266	8.4	0.035
Bw15	13	14.8	566	17.9	0.076
Bw16	5	5.7	165	5.2	0.491
Bw17	5	5.7	259	8.2	0.068
Bw21	1	1.1	104	3.3	0.015
Bw22	2	2.3	111	3.5	0.403
Bw35	9	10.2	414	13.1	0.074
Bw40	24	27.3	579	18.3	0.007
(N=1016)					
Cw1	3	3.5	49	4.8	0.399
Cw2	15	17.0	96	9.4	0.074
Cw3	33	37.5	346	34.1	0.293
Cw4	8	9.1	139	13.7	0.145

Results

Table I shows the results. Five antigens (HLA Aw19 -B8 -B27 -Bw40 and -Cw2) showed increased values in the present patients ($0.05 > P > 0.01$) while two antigens showed decreased values (HLA A11 and -B12). If the *P* values are corrected by multiplication by the number of studied properties (27) then the differences are not significant.

The first patient to be studied was one of a monozygotic pair of twins both of whom suffered from giant cell arteritis (Kemp 1977: the twins are only counted as one patient in the present material). Further two cases of familial occurrence of the disease complex were observed. A 77-year-old woman included in the present material had a sister (deceased) who had developed severe temporal arteritis with bilateral loss of vision some seven years earlier. One 70-year-old woman also included in the material had an older sister with polymyalgia rheumatica, but no histologically verified arteritis. Information was obtained regarding the above relatives from the hospital to which they had been admitted.

Discussion

HLA B8 has previously been demonstrated as occurring more frequently in patients with temporal arteritis and polymyalgia rheumatica. The International HLA and Disease Registry in Copenhagen has incorporated the present data in a new combined analysis (1979). It was found that for HLA B8 in 222 patients with giant cell arteritis (the 9 patients of Terasaki *et al.* are not included) there was a relative risk of the disease in the antigen bearers equalling 1.76 ($P = 0.003$). Svejgaard & Ryder (1977) state, however, that the significance limit in connection with combined analyses should be as low as 0.0004 in order to avoid false associations. Thus it is only an indication.

The present material includes 19 patients with the clinical diagnosis of polymyalgia rheumatica. It was impossible to determine retrospectively how many of the patients with clinical temporal arteritis suffered initially from symptoms of polymyalgia. Both of the diseases are in all probability cardinal manifestations of a generalized giant cell arteritis (Sørensen & Lorenzen 1977).

The frequency of giant cell arteritis in the Danish population is increasing (Kemp 1977) but awareness of the disease has also increased since 1934 (Horton *et al.* 1934). Classical hereditary studies are not possible, as the disease has only been registered for the last 40 years. Further, very few suffer from the disease before the age of 50 years. Liang *et al.* (1974) have, in connection with their report of four pairs of closely related patients, suggested that there are unknown genetic factors which predispose elderly individuals to polymyalgia rheumatica and temporal arteritis. In the present it has been impossible to demonstrate any definite connection between such factors and the HLA system.

Acknowledgment

The authors wish to thank the medical and ophthalmological departments in Esbjerg and Kolding as well as the medical departments of the hospitals in Fåborg, Odense and Ribe all of which have kindly put patients at the disposition of the authors for study.

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THE COLOUR OF THE OPTIC DISC VARIATION WITH LOCATION OF ILLUMINATION

BY

PER NELLEMANN SORESEN

The variation of the colour of the optic disc was examined with a slit lamp and contact lens in 200 normal subjects simply by moving the image of the slit across the optic disc. This event was photographed in 15 subjects and colour densitometry was performed. The temporal part of the pale central excavation changes colour to red when illuminated nasally and vice versa.

The colour change could not be provoked in subjects with normal discs with flattening of the temporal disc sector. The whole neuroretina reddens the whole way round when the slit hits the optic disc border and dependent on fundus pigmentation slight redness was produced even when the slit was placed outside the disc in the neighbourhood of the border.

Keywords: central excavation - colour change - microdensitometry - optic disc colour

The colour of the optic disc is still an important clinical landmark in ophthalmology since it is explicitly correlated to the visual function (Cogan 1966, Walsh & Hoyt 1969). It is well known that the colour of the optic disc changes with the colour temperature of the light source (Miller & George 1978) and the age of the lens (Said & Weale 1959, Snyder 1964). It is also known that colour changes with the incident angle from the light source. Since the importance of this fact is only briefly mentioned in the literature (Kronfeldt 1967, Schwartz 1973) we have studied this phenomenon with the aid of a slit lamp and a contact lens simply by moving the image of the slit across the optic disc. In 15 cases photographs of this procedure were analyzed in a densitometer.

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Materials and Methods

200 normal subjects (age 6-87 mean 42 refractive error less than ± 3 diopters) had both of their optic discs examined in the Haag Streit or Rodenstock slit lamp employing a Goldmann fundus contact lens. When the image of the slit was moved horizontally or vertically across the disc the colour (redness) of the illuminated area and of the rest of the disc was noted as well as the point at which the disc first begins to redden when the slit was moved towards and across the border.

Fifteen cases with cup/disc ratio from 0.2 to 0.9 (estimated by colour contrast) were photographed (Zeiss photoslitlamp magnification $\times 11$ Flash setting IV 350 Wsec slit 0.5 mm aperture 32 slit 3 Kodachrome b-4). The transmission density of the diapositive was measured on the temporal slope of the optic disc excavation corresponding to an area with diameter 100μ . The density was read on a Reichert microdensitometer in the green (540 nm) and red (640 nm) locus where the luminosity is highest and where the unwanted absorption from overlapping between the colour forming pigments is lowest. The density in the blue was not measured since it could be demonstrated in white areas only. The differences between the densities at 640 nm and 540 nm is taken as an arbitrary index of the red colour. The density at 640 nm denotes the colour hue and the density at 540 nm denotes the correction factor for variation in exposure.

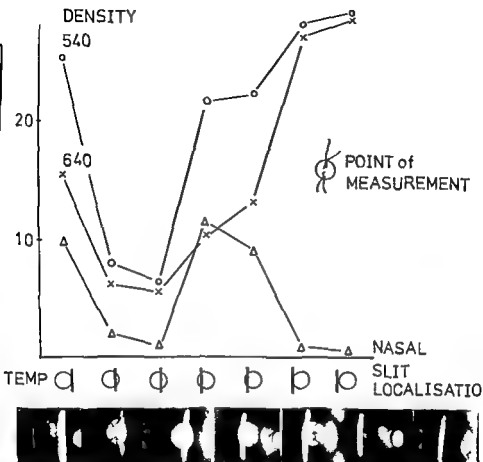
Thus linearity exists between density and the logarithm to the exposure in the proper exposure range of the film for both green and red and furthermore the characteristic curves for green and red have practically identical slopes. Variations in exposure will therefore affect the densities in the same direction. The linearity requirement is not totally fulfilled in the bright parts of the film a fact that is considered of minor importance.

Results

Slit illumination of the optic disc results in a light spreading effect of the whole neurorim i.e. the peripheral part of the non excavated part of the optic disc. This happens as soon as the slit image hits the optic disc border.

In most subjects (92%) also the central physiological pallor turns red on the contralateral side of the optic cup. Thus illumination nasally causes reddening temporally and vice versa. Most effective was disc illumination from the nasal side due to the larger area of the optic cup and perhaps due to a more bank like neurorim on the nasal side.

In 6% of the subjects the excavation only glows when illuminated nasally but not temporally. These subjects had temporal flat bright but normal optic discs with nasal displacement of the vessels.



The redness of the central excavation as an illuminated slit moves stepwise across the left optic disc. Black and white prints of the colour diapositives in which density measurements was made is seen below. The red \times — \times (640 nm) and green \circ — \circ (>40 nm) density is given (arbitrary units). The difference Δ — Δ is an index of the redness.

In all subjects the red glowing could also be provoked by moving the slit in the vertical direction.

No difference between left and right eye could be demonstrated in any subject.

In 30% of the subjects the optic disc begins to redden before the slit image reaches the border. These subjects were predominantly blonds with sparse fundus pigmentation. Thus 41% of the optic discs in blonds reacted in this way compared



Fig 2

Slit lamp photo of the optic disc. The central excavation is white at the temporal side but red at the nasal side

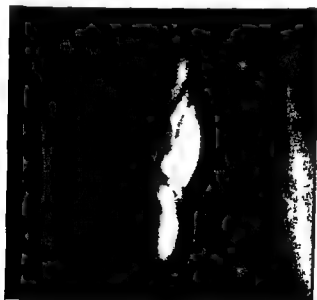


Fig 3

Slit lamp photo of the same optic disc. The central excavation is now red temporally. No light spreading outside the optic disc is seen

to 19% of the optic discs in dark haired. However the reddening was always most pronounced when the slit image reached the border of the neurorim.

Fig 1 demonstrates the colour change of the temporal part of the optic cup when the slit image is moved across the optic disc in a typical record of a subject with well developed optic cup. The mean redness of the temporal excavation when the slit image is placed on the nasal neurorim is 0.96 (range 0.71-1.10 \pm 15). The redness is given by the numerical difference between the densities of the film at 640 nm and 540 nm (arbitrary units).

From Fig 1 it is seen that the optic cup reddens with slit illumination both on the temporal border and also by nasal illumination.

Fig 2 and 3 are colour prints of diapositives of the colour change of the central excavation when the slit image is placed temporally (Fig 2) and nasally (Fig 3). Light spreading in the retina is minimal compared to that of the optic disc.

Discussion

This investigation has shown that the optic disc colour depends on the location of the incident light on the disc. The demonstrated colour change of the physiological excavation of the disc from pale to red when the slit image is moved across the disc is puzzling. Quigley & Anderson (1977) in a histopathological study on monkey eyes with descending optic atrophy considered the optic nerve fiber bundles and their adjacent glial columns as optic pathways. According to them light is transferred in the optic disc longitudinal to the nerveous fibres and adjacent glial cells and later diffused among the capillaries from which the reflected light takes its pink colour. The contralateral reddening of the central excavation however is difficult to explain in this way. On the other hand Gloster (1972, 1973) in his study of optic discs onto which a grid shadow was projected through a fundus camera explains the light transfer of the optic disc tissue as an effect of a diffusing of light projected onto it.

Still the nature of the colour change from pale to red of the contralateral part of the central excavation is obscure. This phenomenon might be either a guidance or a reflection of red light originating from the vascular structures of the disc tissue. Another possibility could be that the colour change is due to an unveiling of underlying vascularity hitherto hidden by veiling reflections from tissue in front of it. Just as the vascularity of the central excavation and apparently pale temporal parts of the disc is disclosed by fluorescein angiography (Hayreh 1969, 1972).

When the disc has a borderline surface two possibilities exist dependent on the angle of incidence. Light can either be reflected or refracted. Reflection can be

specular or scattered according to the properties of the surface. In transparent media refractive index differences determine the degree of refraction. Thus the redness of the optic disc must depend on whether light is scattered or reflected from the surface or penetrates the tissue until it is absorbed or finally reflected from the lamina cribrosa.

The optical properties of the optic disc are not known in detail and need further investigations.

The described dependence of optic disc colour on different location of the illumination of the disc has great clinical importance. Schwartz (1973) advocates examination of the optic disc to be done with the aid of a contact lens and the slit lamp. For example with the direct ophthalmoscope the optic cup is estimated larger.

Better understanding of the colour of the optic disc colour is achieved when considering the light reflected from the optic disc tissue as an information on what transformation the light undergoes when it strikes the optic disc texture.

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PULSED DOPPLER ULTRASOUND FOR MEASURING BLOOD FLOW VELOCITY IN THE HUMAN OPHTHALMIC CIRCULATION

BY

**SIGMUND KVERNES SIGMUND BLIKA JAN GILTVEDT KNUT MATRE
KJELL KRISTOFFERSEN ARNE GRIP and ALF O BRUBAKK**

This study was performed in order to develop a method for studying blood flow in the ophthalmic circulation. Using a pulsed doppler system utilizing an ultrasonic frequency of 10 MHz blood flow velocities have been measured in the ophthalmic artery and in the arteries behind the eyeball (lateral posterior ciliary arteries) in 40 normal subjects. The mean of the peak systolic velocities were 14 ± 6 cm/s in the ophthalmic artery and 14 ± 3 cm/s in the lateral posterior ciliary arteries. We conclude that blood flow velocities can be measured in defined vessel areas in the orbit.

Key words: ophthalmic artery – posterior ciliary arteries – blood flow velocities – pulsed doppler ultrasound

Using fluorescein photographic and photometric techniques blood flow in retinal arteries can be studied (Hickam & Frayser 1966 Niesel & Gassmann 1972).

Relative changes in blood flow to the eye in cases of carotid stenosis have been studied using ocular pulse amplitude (Hørvén et al 1971). Only indirect measurement of blood flow can be performed in this way as changes in ocular volume and compliance will influence the result.

Methods based on continuous wave ultrasound and the doppler principle have been proposed for studying ocular blood flow (Tokoro 1972 Yamamoto 1973).

These methods do not permit range resolution and therefore can not give a defined sample volume with respect to depth.

The method presented in this paper has been based on pulsed doppler ultrasound which permits range resolution and hence measurement of blood flow velocities of selected depths. Furthermore, the size of the sample volume has been experimentally determined. By this method it is possible to measure blood flow velocities more accurately in the ophthalmic and the posterior ciliary arteries.

In this study the feasibility of the method has been demonstrated and the reproducibility of the measurements as well as the range of normal velocities have been documented.

Methods

When ultrasound is transmitted into the body, the soundwaves are scattered from inhomogenities in the tissue and in the blood. The frequency shift (doppler shift f_d) in reflected waves is proportional to the velocity (v) of the scatterers according to the formula

$$f_d = 2 f_0 \frac{v}{c} \cos \theta$$

where f_0 is the frequency of the transmitted soundwaves, c is the sound velocity in the tissue and θ is the angle between the soundbeam and the path of the scatterers.

By pulsing the transmitted waves and detecting the backscattered soundwaves at a given time interval after transmission, depth resolution is achieved (Baker 1970).

The ultrasonic doppler velocity meter used in this study has been described in detail (Angelsen & Brubakk 1976) and only its main features will be given here.

The ultrasonic frequency is 10 MHz. The repetition frequency was 19.5 kHz and a pulse length of 3 microseconds gives a sample length of 1.6 mm. The ultrasonic transducer acts both as a transmitter and as a receiver. The backscattered ultrasound is split into two channels by a quadrature demodulator in order to separate forward from backward flow.

In order to remove signals from slowly moving tissues, a high pass filter with a cut off frequency of 290 Hz was used. The low pass filter has a cut off frequency of 9.3 kHz to remove high frequency noise.

Using analogue estimators described earlier (Angelsen & Brubakk 1976, Brubakk et al. 1977) the mean and maximum velocity in the sample volume can be displayed. However, due to the poor signal/noise ratio when measuring from the posterior ciliary arteries, analogue estimation is difficult and the received signals were analyzed using a frequency analyzer. This instrument is based on a z-chirp algorithm implemented by analogue shift registers (Recon Corp., Sunnyvale, USA).

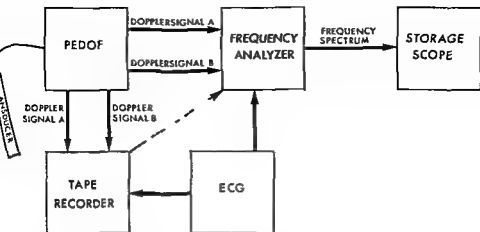


Fig 1

Schematic view of the signal processing equipment PEDOF Pulsed Echo-Doppler Flow meter as developed at the Division of Engineering Cybernetics University of Trondheim

In order to improve the time resolution of the received signals curves from about 10 cardiac cycles were superimposed on an oscilloscope screen using the subject's electrocardiogram as a trigger. The maximum velocity has been given by the envelope around the frequency spectrum.

A schematic representation of the equipment used is shown in Fig 1.

Initially we tested a transducer fastened inside a gonioscopic lens hoping that this would facilitate aiming of the ultrasonic beam by simultaneous observation of the cornea through a slit lamp. However this was not the case firstly because of an avoidable pressure on the cornea secondly because of reduced mobility of the transducer. All measurements in this paper have therefore been done with a dish-shaped transducer made of lead zirconate titanate (Trade Mark PZT 5A, Vishay Clevite). The transducer is quadratic (3 mm) and is backed by araldite. The transducer has an acoustic output power of 28 mW/cm², an efficiency of 12%, a phase distortion of 2% and an impedance of 17Ω.

The output power of the transducer was measured by transmitting the ultrasonic beam into an absorber and detecting the ultrasonic energy by a sensitive balance weight (Mettler H 1 L 52). The principle of this method had been described elsewhere (Kosoff 1965, Matre 1979).

The acoustic field from the transducer was measured in water (Engan 1978). The field was detected by another 10 MHz transducer having a small aperture (0.5 mm) in front of it. The rod transducer was scanned in the x-direction and in the

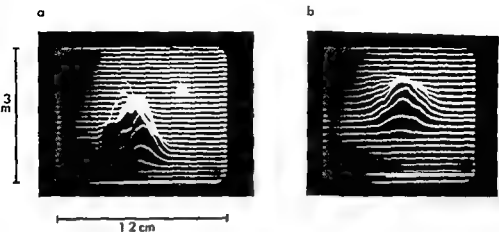


Fig 2

A three-dimensional representation of the ultrasonic field of the rod-shaped transducer in water. The field from the 10 MHz transducer is detected by scanning a 10 MHz rod transducer of same type with a 0.5 mm aperture in front, in a plane 90° to the beam: a) 1 cm from the crystal b) 3 cm from the crystal.

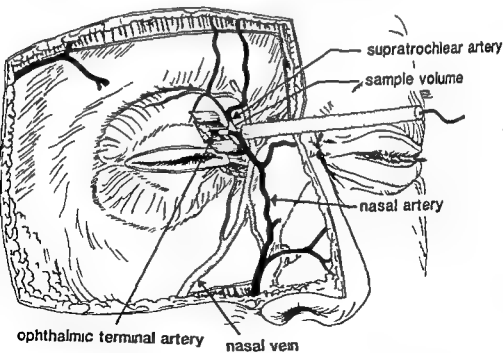


Fig 3

End branches of the ophthalmic artery. The orientation of the transducer and an estimation of the sample volume is shown about 8 mm below skin surface. Redrawn from W. Kahle et al. (1978).

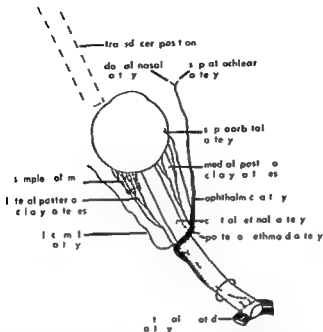


Fig 4

The most common pattern of some of the branches of the ophthalmic artery. The sample volume is located lateral to the optic nerve 24 to 28 mm behind limbus cornea corresponding to the lateral main bundle of the posterior ciliary arteries. The size of the volume is estimated to be 4.6 mm long and 4 mm in diameter. The direction of the transducer is indicated parallel to the optic nerve. Redrawn after Hayreh S S (1976)

y-direction simultaneously. Fig 2 shows a three-dimensional representation of the sound field in a plane 90° to the ultrasonic beam in water at two different depths.

Blood flow velocity was measured in 40 subjects with no history of cardiovascular or eye diseases: 16 women and 24 men (mean age 36 years, range 15–70 years).

Nobody was using any drug at the time of the investigation. The procedure was carefully explained to every subject and consent for doing the study was obtained. All measurements were performed with the subjects in a supine position from the following sites: Left and right ophthalmic artery and left and right arteries behind the eyeball (later called lateral posterior ciliary arteries).

In order to measure flow velocities in the ophthalmic artery the transducer was positioned on the nasal part of the closed upper eyelid. Using methylcellulose as a coupling agent it is possible to measure without exerting any pressure on the tissue. The transducer was moved until maximum velocity was obtained.

The strongest signals were found at approximately 8 mm depth in all subjects. Probably this will occur just before the division of the ophthalmic artery into the supratrochlear and the dorsal nasal artery (Hayreh *et al.* 1962). The approximate location of the sample volume in relation to the vessels can be seen from Fig. 3.

In order to measure the velocities in the lateral posterior ciliary arteries eye movements must be eliminated; therefore the subjects were asked to focus a black spot in the ceiling. The cornea was anesthetized with oxybuprocaine chloride 0.4% eye drops. To avoid sound absorption in the cornea and in the lens the transducer was placed at the limbus cornea in a direction approximately parallel to the optic nerve axis (Fig. 4) and the position was changed until the strongest signals were detected, usually from a depth of 26 mm behind the corneal limbus.

Adequate signals, however, could be detected at depths from 24 to 28 mm. Using methylcellulose no pressure was needed to obtain acoustic contact with the eyeball.

In order to demonstrate changes in flow following alteration of the intraocular pressure, measurements were performed using different weights on the cornea (35–120 g).

Results

Typical velocity spectra from the common carotid artery, the ophthalmic artery and the lateral posterior ciliary arteries are shown in Fig. 5. Each spectrum is a superimposition of about 10 cardiac cycles from a normal subject, 30 years of age.

The peak systolic velocities found in the ophthalmic artery and the lateral posterior ciliary arteries in 40 normal subjects have been shown (Table 1).

The mean of the peak systolic velocities have been grouped according to age. No significant difference in maximum systolic velocities were found with increasing age. The form of the maximum velocity curve, however, changed with increasing age (Fig. 6).

In Fig. 7 histograms of the peak systolic velocities have been shown. The velocities ranged from 21 to 50 cm/s in the ophthalmic arteries and from 10 to 25 cm/s for the lateral posterior ciliary arteries.

In Table II the mean of the peak systolic velocities from each of 9 normal subjects measured 10 times, weeks apart, is presented.

Fig. 8 shows the effect on blood flow velocities in the lateral posterior ciliary arteries when intraocular pressure was changed. The velocity spectrum in Fig. 8a was recorded at normal intraocular pressure.

In Fig. 8b and 8c the intraocular pressure was increased for 30 seconds by placing an external weight, 35 g and 60 g, respectively, on the cornea. With increasing intraocular pressure the maximum flow velocities were reduced, especially in the diastolic part. After release of the pressure (Fig. 8d) the velocity spectrum was

slightly increased as compared to Fig. 8a where normal flow conditions were to be expected. Weights of 90 g and 120 g were also tried. However, no flow could be registered in the posterior ciliary arteries.

In the ophthalmic artery the change of intraocular pressure led to no measurable velocity changes.

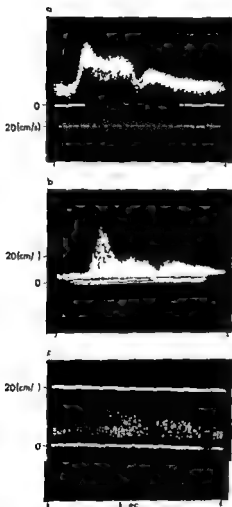


Fig. 5

Velocity spectrum averaged over about 10 cardiac cycles for a) the common carotid artery 13 mm below the skin surface b) the ophthalmic artery 8 mm below the skin surface c) lateral posterior ciliary arteries 26 mm behind limbus corneae

Table I

Mean SD and variation coefficient of the peak systolic velocities in the ophthalmic arteries 8 mm below skin surface and in the arteries 26 mm behind the corneal limbus (lateral posterior ciliary arteries) from 40 normal subjects grouped according to age

No	Age range	Mean SD and variation coefficient of the peak systolic velocities (cm/s)					
		Ophthalmic artery		Lateral posterior ciliary arteries			
		Right side	Left side	Right side	Left side	Right side	Left side
9	(15-25)	36 ± 8	33 ± 8	22%	24%	16 ± 2	15 ± 3
8	(26-30)	34 ± 7	34 ± 5	20%	15%	13 ± 1	13 ± 1
8	(31-35)	32 ± 4	33 ± 4	13%	12%	14 ± 2	14 ± 1
7	(36-45)	37 ± 7	36 ± 6	19%	17%	14 ± 3	16 ± 5
8	(above 46)	35 ± 8	34 ± 8	22%	23%	14 ± 2	16 ± 4
40	(15-70)	34 ± 6	34 ± 6	18%	18%	14 ± 3	15 ± 3
						21%	20%

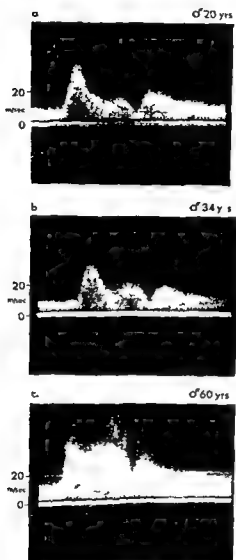


Fig 6

Velocity spectrum from the ophthalmic artery of normal subjects of different age a) male 20 years b) male 34 years c) male 60 years

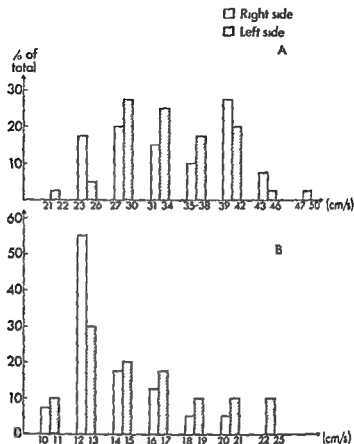


Fig 7

Distribution of the peak systolic velocities in A) ophthalmic artery B) lateral posterior ciliary arteries

Discussion

Using continuous wave ultrasound for measuring blood flow (Tokoro 1979, Yamamoto 1975) velocities in all vessels within the sound beam will be measured simultaneously. Our measurements were performed with pulsed ultrasound giving range resolution and thus the possibility of interference from undesirable vessels will be minimal.

In this study we have demonstrated that it is possible to measure blood flow velocities in defined parts of the ophthalmic arteries and in the posterior ciliary arteries reproducibly in normal persons.

Table II

Mean SD and variation coefficients from the ophthalmic artery 8 mm below skin surface and from the lateral posterior ciliary arteries for three individuals

Subject (Age)	Mean SD and variation coefficient of the peak systolic velocities (cm/s)					
	Ophthalmic artery		Left side		Right side	
	Right side	Left side	Right side	Left side	Right side	Left side
JC (28)	57 ± 4	53 ± 3	108 ± 9	87	131 ± 13	107
SB (36)	47 ± 2	40 ± 1	131 ± 9	57	146 ± 20	157
SK (33)	51 ± 3	55 ± 1	123 ± 10	107	139 ± 14	117

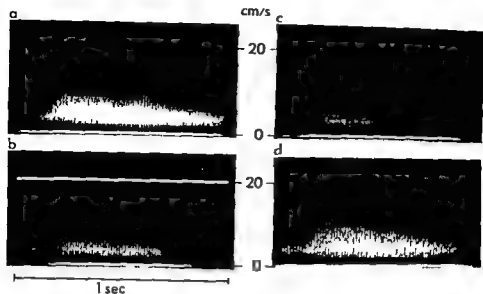


Fig 8

Velocity changes in the lateral posterior ciliary arteries following raised intraocular pressure achieved by placing different weights on the corneal surface for 30 seconds (Superimposition of about 10 cycles) a) Normal velocity spectrum b) 35 g c) 60 g d) after removing weights

The variation coefficients are rather great (20%) (Table I) and of the same order for the ophthalmic arteries as for the posterior ciliary arteries

Repeated measurements on the same person showed a variation coefficient of about 10% (Table II). The difference between the variation coefficients of these two groups is probably explained by anatomical variations.

The angle between the blood stream and the position of the sample volume can not be known for certainty. The posterior ciliary arteries consist normally of two main bundles, one on the temporal and one on the nasal side of the optic nerve (Hayreh & Dass 1962; Hayreh 1962). With our procedure the angle between the sound beam and the blood stream will be small (Fig. 4) and thus the influence on the measured velocities minimal. An angle of $\pm 15^\circ$ for example would reduce the measured velocity only in the order of 3%.

The course of the ophthalmic arteries shows greater variation. Signal noise ratio however is good and finding maximum signals corresponding to a minimum angle is easy.

The sample area is given by the intensity of the sound, which is proportional to the field in the second power.

The diameter of the effective sample area in our case will be less than 1 mm (Fig. 2).

When measuring behind the eyeball with a cylindric sample volume with a length of 4.6 mm and a diameter of less than 4 mm the frequency spectrum of the backscattered sound will be a superimposition from several vessels. We have assumed that the main contribution will be from the lateral posterior ciliary arteries. Interference from other branches of the ophthalmic artery is possible. As the blood flow in the central retinal artery is very small compared to that in the ciliary arteries (Alm & Bill 1973) this contribution is probably negligible. Other branches of the ophthalmic artery will according to their anatomical course usually not interfere but may in case of vascular anomalies play a role.

We have measured the flow velocities in the terminal trunk of the ophthalmic artery and in the lateral posterior ciliary arteries at different intraocular pressures. This increase in pressure had no effect on the flow velocities in the central artery. Changes in the velocities in the lateral posterior ciliary arteries were considerable (Fig. 8). In particular the diastolic part of the cycle disappeared with only 35 g weight on the cornea.

The form of the maximum velocity curve from the small arteries like the ophthalmic artery and even from the lateral posterior ciliary arteries has been well preserved compared to the common carotid artery (Fig. 5).

The shape of the maximum velocity curve changed with increasing age showing a significant increase in the second systolic velocity peak in the older persons (Fig. 6). This increase is probably due to structural changes in the vessel wall.

With our instruments it is possible to measure velocities above 2.3 cm/s corresponding to the cut-off frequency of the high pass filter which is necessary to remove noise from slowly moving targets.

When using ultrasound doppler to measure blood flow velocities uninvvasively one problem is to reproduce the position of the sample volume from one measurement to the other. With knowledge about the topographical anatomy and an exactly known size of the sample volume, as is the fact using pulsed ultrasound adequate reproducibility seems possible.

When using ultrasound for measuring in the orbit the possibility of damaging effects in the eye must be considered. So far no definite safety limits of ultrasound in ophthalmology have been determined. Experiments with pulsed ultrasound in rabbits (Barnett & Kosoff 1977) using a 7.5 MHz focused transducer with a mean power output of 160 mW/cm² on retina and 20 mW/cm² on the cornea for 30 min showed no morphological changes.

In this study the mean power into the eye was 28 mW/cm². Exposure time was kept as short as possible and did not exceed 10 min in any of the subjects. No adverse effects were observed.

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Examples of references

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